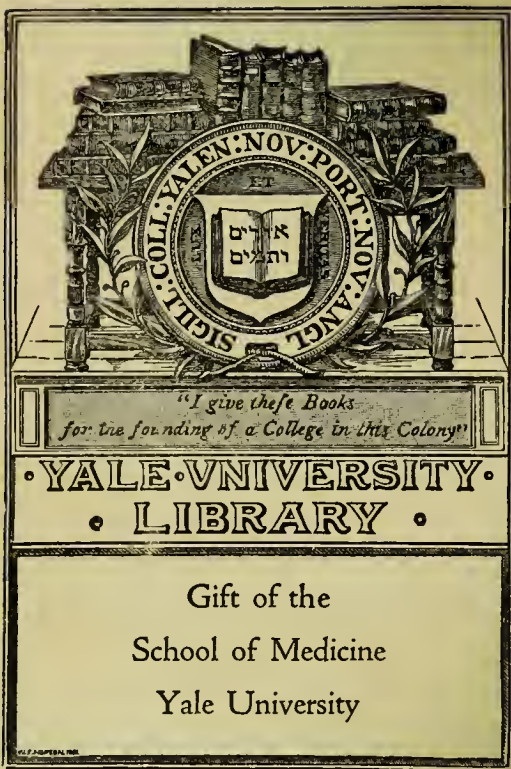


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# SERUMS, VACCINES AND TOXINS IN TREATMENT AND DIAGNOSIS

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# CONTENTS

CHAPTER	PAGE
1. IMMUNITY AND RESISTANCE TO DISEASE . . . . .	1
2. PREPARATION AND ADMINISTRATION OF SERUMS . . . . .	41
3. BACTERIAL VACCINES: THEIR PREPARATION AND ADMINISTRATION . . . . .	59
4. SERUMS AND TOXINS IN DIAGNOSIS. . . . .	76
5. DIPHTHERIA . . . . .	94
6. TETANUS . . . . .	138
7. SNAKE-BITE . . . . .	157
8. HYDROPHOBIA (RABIES) . . . . .	168
9. SMALL-POX AND VACCINIA . . . . .	182
10. ANTHRAX AND GLANDERS. . . . .	201
11. PLAGUE . . . . .	206
12. CHOLERA . . . . .	220
13. ENTERIC FEVER. . . . .	229
14. DYSENTERY AND OTHER BACILLARY INFECTIONS . . . . .	257
15. TUBERCULOSIS . . . . .	270
16. TUBERCULOSIS (concluded) . . . . .	311
17. LEPROSY; STREPTOTRICHOSIS; RINGWORM . . . . .	345
18. AFFECTIONS DUE TO STREPTOCOCCI . . . . .	349
19. OTHER INFECTIONS DUE TO COCCI . . . . .	373
20. CATARRHAL AFFECTIONS . . . . .	398
21. DISEASES DUE TO PROTOZOA . . . . .	405
22. MALIGNANT TUMOURS . . . . .	422
APPENDIX: VARIOUS CONDITIONS TREATED WITH SERUMS, ETC. . . . .	434
INDEX . . . . .	447

## LIST OF ILLUSTRATIONS

FIG.	PAGE
1. Action of hæmolytic serum . . . . .	7
2. Effect of heat on hæmolytic serum . . . . .	8
3. Addition of complement from normal serum to heated immune serum . . . . .	9
4. Diagrammatic representation of agglutination . . . . .	14
5. Diagram illustrating "side-chain" hypothesis . . . . .	23
6. Diagrammatic representation of hæmolysis . . . . .	24
7. Diagram of cell with numerous side-chains produced by stimulation with toxin . . . . .	26
8. Diagram illustrating fixation or deviation of complement . . . . .	39
9. All-glass serum-syringe . . . . .	49
10. The opsonic cycle . . . . .	70
11. Opsonic index, showing rapid fall . . . . .	70
12. Opsonic index, showing less marked negative phase . . . . .	71
13. Opsonic index, showing oscillation about normal level . . . . .	71
14. Opsonic index, showing immediate appearance of positive phase . . . . .	71
15. Measuring-pipette for opsonin-estimation . . . . .	85
16. Edge of blood-film preparation . . . . .	86
17. Diagram illustrating process of saturation of diphtherial toxin with antitoxin . . . . .	103

## LIST OF CHARTS

CHART	
1. Illustrating effects of Chantemesse's serum . . . . .	237
2. Illustrating effects of Chantemesse's serum . . . . .	238
3. Illustrating efficacy of a second brand of antistreptococcic serum after failure of the first . . . . .	357

## PREFACE TO THE THIRD EDITION

THE period—little more than six years—that has elapsed since the publication of the second edition of this book has been so fruitful in research, and has witnessed such notable advances in methods of diagnosis and treatment, that an exhaustive revision of its contents was found necessary, entailing the addition of nearly one hundred pages of new matter. Some rearrangement of the subjects dealt with has also seemed advisable.

The most striking advance in the field of therapeutics is undoubtedly the scientific employment of Chemotherapy, which has resulted from the noteworthy investigations of the late Professor Ehrlich and his pupils; and although the inclusion of drug-treatment in a volume professedly devoted to the use of serums and vaccines may appear illogical, such is not actually the case, since the lethal action of arsenical compounds and dye-stuffs upon certain protozoal parasites depends upon principles fundamentally identical with those governing the action of specific serums upon bacteria. We have therefore deemed it advisable to devote some space to a consideration of this subject.

Experience gained during the present European War has vindicated in a most convincing manner the value of prophylactic vaccination against typhoid fever—a procedure which appears to afford a protective immunity against this disease, second only in efficiency to that given by Jennerian vaccination against small-pox. Other lessons taught by the campaign are less definite; thus, in connection with the use of antitetanic and of antimeningococcic serum, opinions are still divided on such questions as the size and frequency of the doses used, the method of administration, and so forth. On these points, as elsewhere throughout the book,

we have collated the published experience of others, as well as indicated the conclusions drawn from our own observation.

Other points to which we may direct attention are the extended use of the complement-fixation reaction, not only in the form of the well-established Wassermann test for syphilis, but also in the diagnosis of other infections; the attempt to correlate certain physical phenomena with this reaction; and the present trend of opinion on the use of tuberculin in cases of pulmonary tuberculosis, on which subject the confident dicta and heroic dosage of the enthusiasts may have temporarily dazzled the unwary practitioner of medicine.

It will no doubt occur to the critical reader that somewhat brief mention is made of the dosage of ordinary bacterial vaccines, whether autogenous, stock or sensitized. The omission is intentional, for the reason that the study of the standardization of vaccines is still in its infancy, and consequently it is almost impossible to compare the effective doses of vaccines bearing the name of the same micro-organism but emanating from different laboratories. This fact is especially noticeable in connection with vaccines prepared by the different commercial houses.

A word of warning may perhaps not be out of place in regard to the intrusion of this commercial element into the field of bacterial therapeutics, and, on the other hand, as to the need for careful clinical observation of cases undergoing treatment by such remedies.

W. C. B.

J. E.

*March, 1916.*



# SERUMS, VACCINES, AND TOXINS

## IN TREATMENT AND DIAGNOSIS

### CHAPTER I

#### IMMUNITY AND RESISTANCE TO DISEASE

**Acquired resistance.**—The problem of the nature of disease and of the manner in which living bodies resist and recover from its attacks has exercised the minds of mankind since very early times, and the fundamental truth that in the case of certain diseases one attack protects against subsequent infection was long ago discovered. The first attempt to utilize this principle for prophylactic purposes was made in the East in the form of inoculation against small-pox, a mild infection being produced by inoculation with matter derived from a pustule, in order to ward off danger of subsequent infection. Shortly after the introduction of this procedure into the West of Europe, Jenner's great discovery that vaccination with cow-pox was equally efficacious as a protection and practically free from risk, laid the foundation of our knowledge of therapeutic inoculation. It was only, however, after the discovery of the minute living agents which cause infective diseases had been rendered possible by improvements in optical science that further advance in this field was made. When bacteria were recognized as the cause of most infective diseases, and their mode of action by the secretion of poisons or toxins was ascertained, research into the problem of our means of resistance to these invaders was stimulated; and from the time of Koch's invention of accurate methods for the separation and identification of bacteria, followed as it

was at no distant date by Behring and Kitasato's work on antitoxins and by Pfeiffer's experiments in bacteriolysis, knowledge of the various factors involved in immunity has steadily advanced.

**Phagocytosis.**—The first important theory of resistance to disease was that of Metchnikoff (1865–84), who studied the behaviour of the white blood-corpuscles (leucocytes) in many of the lower animals, and attributed the destruction of bacteria in the body to the activity of these cells. This was the well-known theory of *phagocytosis* (φαγεῖν, to eat; κύτος, a cell). According to Metchnikoff, the leucocytes attack and devour any invading organisms which they may meet, and thus rid the body of these parasites, just as they may be seen to take into their substance particles of any foreign matter which comes in their way; when they have swallowed and thus destroyed all the bacteria which have gained a footing in the body, the disease necessarily comes to an end.

The careful and minute study carried out by the French observer cannot be too much admired, and there can be no doubt that it contains a large proportion of truth. Thus, the assemblage of leucocytes which takes place at any focus of irritation is almost certainly protective in character; and it has been shown by Kanthack and others that the granules contained in the protoplasm of the leucocytes consist of substances which tend to combat the bacteria and to stop their growth. But in man, at all events, this phagocytic action is not the sole factor in the struggle with the invading germs—possibly not even the most important.

**Protective power of serum.**—Further experiments showed that the serum of the blood, even when all formed elements, such as the corpuscles, had been removed, still exerted in many instances an inhibitory action on the growth of micro-organisms (Nuttall, Büchner). There must, therefore, be present in the plasma some substance of a protective nature, and to such hypothetical substances Büchner gave the name “alexines.” It is by means of

such alexines that destruction of bacteria is in many cases brought about; and it is by other chemical substances circulating in the blood that the poisonous products of the organisms are neutralized.

As a result of these discoveries, attention was directed for a time mainly to the chemical contents of the serum and other fluids, and the importance of the process of phagocytosis was seriously called in question. A whole series of peculiar properties possessed by the serum of immunized animals was brought to light, and was used to support the "humoral theory" of immunity. Here, however, as in many other instances in which opposing theories have been hotly upheld and attacked, time has shown that each side in the controversy had grasped a portion of the truth, but declined in the heat of conflict to recognize the other portion which was defended by their antagonists. The upholders of the phagocytic hypothesis satisfactorily proved that in many important affections the serum alone was ineffective in destroying bacteria and that the action of leucocytes was essential for the process of defence, and maintained further that the very chemical bodies which were held by their opponents to constitute the basis of immunity were themselves secreted by the leucocytes of the blood. The discovery of a special group of substances ("opsonins," p. 18) existing in the serum, by which the process of phagocytosis is induced or at least facilitated, has to some extent reconciled the positions of the opposing schools. Thus, so far as is at present known, it would appear that both phagocytosis and bacteriolysis (p. 7) take part in the destruction of bacteria within the body, now one, now the other predominating, according to the nature of the infective agent.

**Resistance of tissue-cells.**—In addition to the fact that protective bodies found in the serum are almost certainly formed by the cells of the blood-forming or other tissues, and to the further fact that certain fixed cells, notably those of endothelial surfaces, possess phagocytic power it can hardly be doubted that the tissues of different

individuals or species have different degrees of power of resistance to the attacks of bacterial parasites. For example, in pulmonary tuberculosis we find that in one case the lung-tissue rapidly breaks down and is destroyed ; in another, little damage of this nature results, but a dense growth of fibrous tissue takes place and effectually localizes the disease, and ultimately repairs the mischief done by the tubercle bacilli. So little, however, is known of this tissue resistance that we need not do more than indicate its existence as an important though incalculable factor in the problem of immunity.

**Antitoxins.**—In the year 1890 Behring and Kitasato published the results of their important researches on the poison of *tetanus* and on the possibility of rendering animals immune to it. These observers proved that it was possible, by injecting animals first with infinitesimal quantities, later with increasing doses, of the toxins of tetanus, to render them immune to the disease. The animals thus treated were able to support with impunity doses of the tetanus poison many times as great as would suffice to kill an ordinary non-immunized animal of the same species. If the serum of an immunized animal were mixed with an equivalent amount of the poison and injected into a non-immune animal, no ill effects were produced ; while the injection of the immune serum itself into a non-immunized animal rendered the latter also resistant to a subsequent dose of the toxin. Finally, if a sufficient quantity of immune serum were administered within a short period of time to an animal previously inoculated with the tetanus bacillus, the disease did not develop.

The same observers, and also Wernicke, shortly afterwards showed that similar possibilities existed with regard to the bacillus of *diphtheria*—that by treating animals with the toxins of this organism a serum could be obtained which was capable of neutralizing the poison, and which also possessed a curative effect on the disease. To the unknown substance in the serum which had the property of

neutralizing the toxin they gave the name of antitoxin. The antitoxic bodies formed in the two cases were not the same; the tetanus antitoxin did not act as an antidote to the poison of diphtheria nor, conversely, did diphtherial antitoxin prove efficacious against tetanus. Each serum was "specific," neutralizing only the poison of the corresponding disease; and this peculiarity has been found to exist in all subsequently prepared "immune serums."

In the light of these discoveries as to the reaction of living animals to bacterial toxins, attention was turned to the effects produced by other organic poisons, and it was shown by Ehrlich that it was possible to immunize animals to the vegetable poisons abrin (from jequirity), ricin (from castor oil), and crotin (from croton oil), which are probably of complex proteid nature, and resemble ferments in their action. In the case of each of these substances it was possible to obtain a specific antitoxic serum, protecting only against its appropriate toxin. Similarly, in the case of snake-venom an antitoxic serum was prepared, of which use has been made therapeutically with some degree of success.

*Chemical nature of antitoxins.*—Examination of the blood of horses used for the preparation of diphtherial antitoxin shows that the globulin content of the serum is increased.<sup>1</sup> Further, if the antitoxic serum obtained from them is fractionally precipitated with ammonium sulphate, it is found that the antitoxin is precipitated with the pseudo-globulin<sup>2</sup>—that portion which is thrown down by semi-saturation with the salt. In animals other than horses (e.g. goats) the antitoxin may be thrown down with the euglobulin precipitate. Hence it has been inferred that the antitoxin is a globulin. This cannot be regarded as definitely proved, since substances are often carried down with precipitates from which they are

<sup>1</sup> This is denied by Ledingham, *Journ. of Hygiene*, 1907, vii. 65, 92.

<sup>2</sup> Pick, *Hofmeister's Beitr.*, 1901, p. 1384.

chemically distinct (e.g. ferments). Proescher<sup>1</sup> believes that antitoxins are non-albuminous, but their non-dialysable character points to the probability of their molecules being large in size, and therefore complex in structure, as are all the albuminoids.

**Antibacterial serum.**—It may here be pointed out that in order to prepare an antitoxic serum it is necessary to obtain the toxin of the bacterium in question for the purpose of injection into animals. In the case of diphtheria and tetanus this is easily done. In the case of many organisms, however, difficulties arise, since their poison is not secreted into culture-media, but remains in the bodies of the bacteria themselves. Such poisons are spoken of as “endotoxins”: it is not certain whether they consist of the actual protoplasmic substance of the bacteria or are separate products, analogous to the poisonous alkaloids formed by some plants (strychnine, morphine). In such instances, if the actual germs are injected into animals, beginning with minute doses of attenuated cultures and gradually increasing until large quantities of virulent bacteria can be tolerated, in most cases a serum is produced which is not antitoxic in the sense of neutralizing the poisons of the micro-organism, but which destroys the bacteria themselves when they are submitted to its action. Such a serum is said to be “antibacterial” or “bactericidal,” instead of antitoxic. Thus, if an animal is injected with cholera vibrios until it is very resistant to these germs, and then a little of its blood-serum is added to a culture of these organisms, the latter are found to undergo degeneration, and finally to be completely disintegrated; but no quantity of this serum will neutralize a lethal dose of the poison of the cholera germ. On the other hand, an antitoxic serum has no effect in preventing the growth of the appropriate organism; for instance, diphtherial antitoxin is a very favourable culture-medium for the Klebs-Löffler bacillus. The process of destruction of

<sup>1</sup> *Münch. med. Woch.*, 1902, p. 1176.



bacteria by an antibacterial serum is called "bacteriolysis," and the property resides not only in the blood-serum, but also in other vital fluids, such as the peritoneal exudate. It is evident that some special substance is produced in the

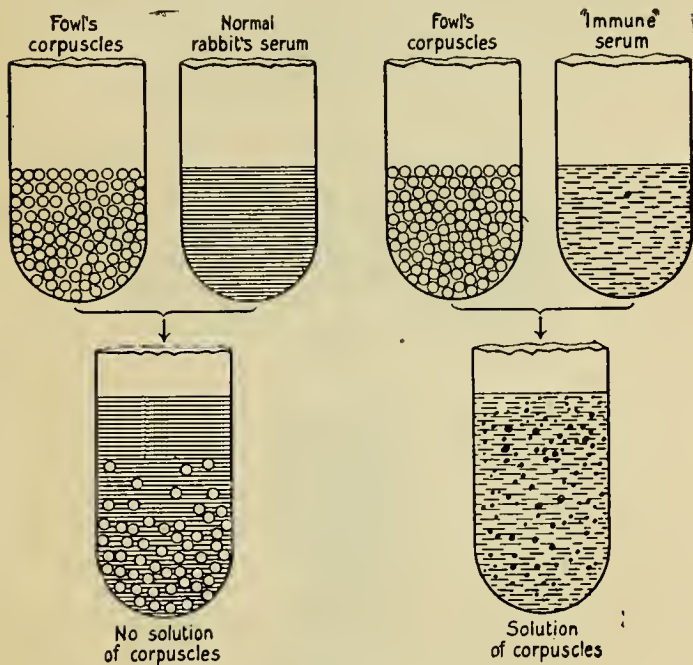


Fig. 1.—Action of hæmolytic serum.

bodies of the immunized animals, which acts as a solvent of the bacterial protoplasm.

**Hæmolysis.**—Further research showed that it is not only bacteria which, by injection into living animals, give rise to the production of substances destructive to themselves. In 1891 Bordet, to whom much of our knowledge of the phenomena of bacteriolysis is due, discovered that, if the blood of one species of animal were injected into

an individual of another kind, the serum of the latter developed the property of dissolving the corpuscles of animals of the former species. Thus, if the blood of a fowl is injected into a rabbit, the serum of the rabbit gains the power of dissolving the corpuscles of fowl's blood when

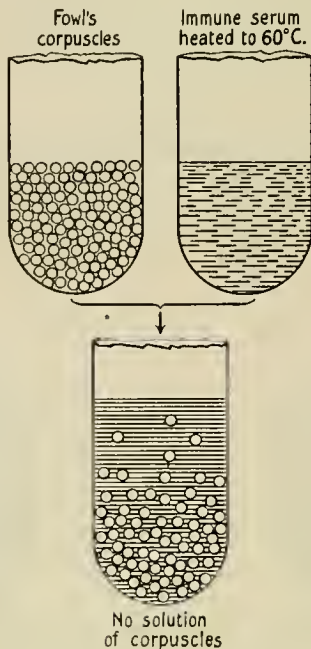


Fig. 2.—Effect of heat on hæmolytic serum (destruction of complement).

added to it in a test-tube. This phenomenon is called “hæmolysis,” and the hæmolytic power is exactly analogous to the bacteriolytic property in the cases previously described.

**Copula and complement.**—Now, if the hæmolytic serum of the rabbit in this experiment is heated to 56° C. or 60° C., it loses its solvent power; but if a little serum from a normal rabbit is added to the heated serum, the property of dissolving the fowl's corpuscles returns to it.



The same occurs in the case of bacteriolytic serum. Thus Pfeiffer showed that in the peritoneal cavity of an immunized guineapig cholera vibrios undergo a process of destruction, and that the same occurs in a test-tube containing serum derived from such an animal. If this serum

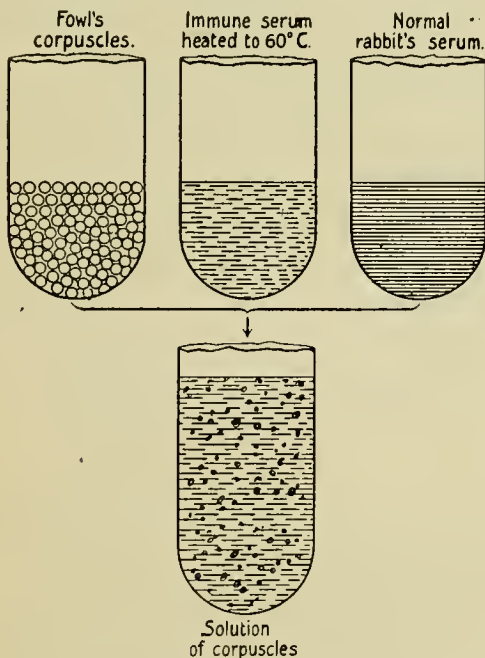


Fig. 3.—Addition of complement from normal serum to heated immune serum.

is heated, it loses its potency. If, however, a little of this heated serum is injected along with cholera germs into the peritoneal cavity of a non-immune guineapig, solution takes place, the peritoneal fluid of the normal guineapig supplying the substance which was destroyed by the heat.

The accompanying diagrams (Figs. 1, 2, 3) will make these statements more clear.

From this it is apparent that in the process of hæmolysis the interaction of two bodies is necessary to bring about the result. One of these—the complement or alexine (*a*)—is present in normal serum, whereas the second—the copula or immune body (*b*)—is only developed in immunized animals. The former (*a*) is quickly destroyed by heat, whereas the latter (*b*) is more stable and is resistant to it. The complement is probably of ferment-like nature, and the process of destruction has been supposed to be one of hydrolysis (Turro).<sup>1</sup>

The multiplicity of names applied to these two bodies by different writers may possibly lead to some confusion. We may tabulate them thus:—

( <i>a</i> ) <b>Complement</b> (always present), called also—	( <i>b</i> ) <b>Copula</b> (developed in im- munized animals), called also—
Alexine.	Intermediary body.
Addiment.	Immune body.
Cytase.	Amboceptor.
	Fixative.
	Mediator.
	Desmon.
	Préparateur.
	Substance sensibilisatrice.
	Sensitizing substance.

Perhaps the words *complement* and *copula* are as convenient as any to denote these substances respectively.

The foregoing experiments do not succeed if the serum and corpuscles be kept at a low temperature (0° C.). If, however, a mixture of hæmolytic serum and corpuscles is made and kept at this degree of temperature, and then the corpuscles are separated from the serum and washed clean by saline solution, it is found that they are now destroyed by the addition of a normal (non-hæmolytic) serum. This shows that the copula or intermediary body has become fixed to the corpuscles in some way, so that these are now “sensitive” to the action of the complement contained in

<sup>1</sup> *Berlin. klin. Woch.*, Sept. 7, 1903.

normal serum. For this reason the copula has been called by French writers the "sensitizer" or "preparator" (*substance sensibilisatrice; préparateur*). Use is made of corpuscles—or of bacteria, for the same occurs in their case also—thus sensitized, for the purpose of experiments, to which further allusion will be made on a subsequent page (*see p. 36*).

The serum of certain animals is found to be actively destructive of the corpuscles of those of another species, without the necessity for any preliminary treatment by injection of the blood-corpuscles derived from the latter. Thus the serum of the eel produces rapid hæmolysis if injected into mammalian animals, and hence is highly poisonous in its action. It is probable that in this case the intermediary body or copula necessary for the action upon blood-corpuscles of a ferment already existing in the mammalian blood is supplied by the serum of the eel. In other cases minor degrees of the same toxicity may be observed, the serum of many animals exerting, without preliminary treatment, a limited degree of hæmolytic action on the corpuscles of other species.

In some diseases, hæmolytic substances may develop in human blood, capable of acting on the blood of another human individual (*isolysins*). The possibility of the existence, in diseases characterized by extreme bloodlessness, such as pernicious and splenic anæmia, of substances of the nature of copulas, which unite with the complement present and thus lead to the destruction of the patient's own blood-corpuscles, opens up an interesting field of speculation; but there is as yet little definite evidence of the existence of such substances (*autolysins*).

**Cytolysis.**—It is found that similar "antibodies" are produced by the injection into a living animal not only of bacteria and blood-corpuscles, but of many other kinds of cells, as, for instance, spermatozoa, nerve-cells, leucocytes, liver-cells, gastric epithelium,<sup>1</sup> etc., provided that such cells

<sup>1</sup> Bolton, *Trans. Path. Soc.*, 1906, lvii. 297.

are derived from an animal of a different species. Serum from an animal thus injected with spermatozoa derived from another species is found to contain a substance (*cytolysin*) capable of destroying the spermatozoa existing within the living body of an individual of the latter species. The question of the possibility of preparing a serum which should be capable of destroying the cells of a tumour, e.g. a cancer, without affecting the normal epithelium, is of interest in relation to the treatment of such disease.

Any substance the injection of which into a living animal induces the formation of a specific antagonistic body is called an *antigen*; and conversely, such an antagonistic substance (*see* p. 29) capable of interacting with its antigen is termed an *antibody*.

**Precipitation.**—Very closely allied to this formation of cytolysins, or substances which are capable of dissolving cells, is the appearance of materials which act in a peculiar way on the soluble albuminous substances contained in serum itself. These are called *precipitins*, and are formed when the serum of one kind of animal or some similar albuminous fluid is injected into the body of another species. Thus, if the serum of, say, a horse is injected into a goat, the serum of the latter acquires the property of forming a precipitate with normal horse's serum. The precipitate is apparently formed at the expense of the "immune serum" (i.e. that of the animal which has received the preliminary injection of foreign albumin), not of the normal serum. It was suggested by Uhlenhuth that use might be made of this fact to constitute a test for different kinds of blood. The possession of such a test for human blood would be of considerable medico-legal value; but unfortunately this particular test is not so absolutely specific as might be wished, since the serum of the injected animal is found to give a precipitate not only with the serum of the actual species of animal used to inoculate it, but also with the blood of closely allied species (e.g. apes and man). Further, it must be borne in mind that the

test is one for distinguishing between albuminous substances derived from different species, and not for blood alone.

Some authorities<sup>1</sup> deny that even this limited degree of specificity exists, finding that a precipitating substance, formed by injecting human blood into an animal—which therefore should act solely on human or anthropoid blood—will react also with that of oxen, horses, sheep, pigs, etc. The most pronounced action is, however, on human blood, and, according to these authors, error may be avoided by diluting the serum. Thus an efficient precipitating serum diluted to 1 in 1,000 will react only with the blood of the animal with which it was prepared. It is possible, therefore, that with this modification the test may still prove to possess a field of usefulness. The age of the blood used—stains on linen, etc.—does not affect the reaction.

**Agglutination.**—The serum of animals treated by injection of the blood of another species possesses, in addition to its destructive action, a power of agglutinating the blood-corpuscles of the latter, i.e. causing them to adhere together. This property is not lost on heating the serum to 55° C. H. Marx and Ehrnrooth<sup>2</sup> found that human corpuscles are agglutinated by the serum of any other animal, and suggested the use of this property as a medico-legal test.

When the serum of animals which have suffered from a bacterial disease, or which have been inoculated with a micro-organism, is added to a culture of the bacteria in question, it causes them to stick together in clumps or masses, instead of floating separately in the culture-fluid (Fig. 4). The best-known instance of this is the reaction produced by the serum of a patient suffering, or who has recovered, from enteric fever, when it is added to a culture of the *Bacillus typhosus*. It is found that if we take a young and vigorous culture of typhoid bacilli in broth, and

<sup>1</sup> Linessier and Lemoine, *Gaz. des Hôp.*, March 27, 1902.

<sup>2</sup> *Münch. med. Woch.*, Feb. 16, 1904, p. 293.

add to it a small quantity of the serum of a patient with enteric fever, the bacilli almost immediately cease their normal active movement, and soon become collected together into clumps. The hypothetical bodies on which this reaction depends are called *agglutinins*. The discovery of this phenomenon is due to Grüber and Durham, and experiments were made with regard to its clinical possibilities by Grünbaum; but Widal first published his results, showing the possible use of the phenomenon as a test for the existence of enteric fever, and the reaction is generally associated

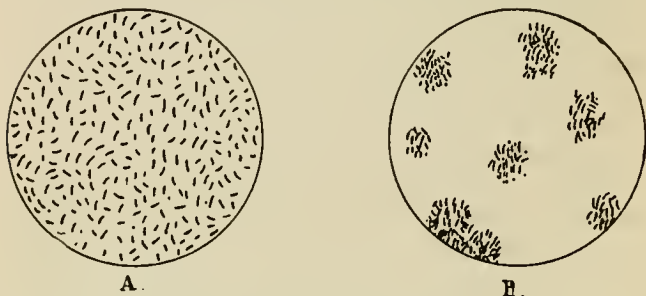


Fig. 4.—Diagrammatic representation of agglutination.

A, Free bacilli; B, bacilli agglutinated.

with his name. Not only are typhoid bacilli agglutinated by their appropriate serum, but the same phenomenon occurs with other organisms, such as the *Micrococcus melitensis* of Mediterranean fever, the bacillus of dysentery (Shiga), the vibrio of cholera, tubercle bacilli, etc.

It was at first thought that the property of agglutinating bacteria was "specific," i.e. that a serum would only clump the particular kind of organism which had been injected into the animal, or which had caused disease in the patient. This appears to be only generally true. On the one hand, it is found that a certain degree of agglutinative power towards many different kinds of bacteria may exist in normal blood, so that inoculation or disease only increases

an already existing property ; on the other hand, it appears that in some cases, at all events, treatment with a particular organism may increase the agglutinative power as affecting other varieties of germs. Thus, in a case quoted by Posselt and Sagasser,<sup>1</sup> it was found that the serum of a rabbit before treatment possessed an agglutinating power against typhoid bacilli in a dilution of 1 : 10 ; against colon bacilli, 1 : 8 ; against cholera vibrios, 1 : 10 ; and against Shiga's bacilli (dysentery), 1 : 5. After inoculation with colon bacilli, the figures rose to—*B. typhosus*, 1 : 150 ; *B. coli*, 1 : 650 ; *V. cholerae*, 1 : 50 ; and *B. dysenteriae*, 1 : 80. Thus treatment with one organism may apparently increase the agglutinative power against a number of others, and hence the property cannot be looked on as quite specific. It is noticeable, however, that the clumping power towards the bacilli injected rose much more rapidly and to a vastly higher point than that towards other organisms.

**Nature of the agglutinative process.**—That the agglutinating power depends on a definite substance present in the serum is shown by the fact that it is possible to exhaust the agglutinin in a specimen of serum by adding a sufficient amount of the bacteria on which it acts. Thus, if we continually add fresh quantities of typhoid bacilli to the serum derived from a patient suffering from enteric fever, there at length comes a time when no further aggregation of the organisms takes place. But such a specimen of serum may still agglutinate other organisms, as, for instance, *B. dysenteriae*. This proves that different substances serve as agglutinins for different species of organisms.

The agglutinins are in all probability not the same as the other antibacterial bodies by which immunity is brought about (bacteriolysins, opsonins, etc.), but in the majority of cases they seem to be developed in the serum *pari passu* with the latter. It has therefore been suggested that the agglutinating power might be used as a criterion of the

<sup>1</sup> *Wien. klin. Woch.*, 1903, No. 24, p. 691.



strength of an immune serum; and Kech considered that in tuberculosis the agglutinative power possessed by the serum is actually an index of the patient's power of resistance. It is probable, however, that no single one of the different bodies produced in the serum of immunized animals can be taken by itself as a measure of the total degree of immunity.

The exact method by which the agglutination of bacteria by their appropriate serum is brought about is not understood. It has been suggested that it is owing to some alteration of their covering membrane, so that they are rendered liable to be wetted by the fluid in which they are floating (Defalle).<sup>1</sup> Bodies which are wetted by a liquid in which they are suspended tend to adhere to one another, while those which are not so wetted tend to repel each other.

A more probable explanation is that some proteid substance is precipitated by the action of the serum and binds the bacteria together in its meshes. It is noteworthy that in old cultures of typhoid bacilli an agglutinative substance passes out into the culture-fluid, so that the addition of a portion of such fluid, freed from organisms, confers on a normal serum the power of agglutinating the bacilli. The relation between precipitins and agglutinins is probably very close.

It would appear that two separate bodies take part in the process of agglutination (analogous to the two required for bacteriolysis, hæmolysis, etc.), one being destroyed by heat, while the other is relatively thermostable. Thus, Bail<sup>2</sup> finds that the serum of a patient suffering from enteric fever loses its agglutinative power if heated to 70° C., but that, if normal serum is subsequently added to a suspension of the bacilli (*B. typhosus*) in this heated serum, clumping will then occur. The thermostable body left after heating has been termed *agglutinoid*. Bacteria

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 595.

<sup>2</sup> *Prager med. Woch.*, 1901, Nos. 32 and 33.



which have been in contact with agglutinoid fail to clump on subsequent addition of agglutinin.

A peculiar phenomenon ("zone of inhibition" or "pro-zone") sometimes observed, viz. that some serums may produce agglutination when diluted but not when concentrated, has been explained on this basis: If there is present a large amount of agglutinin along with a small amount of agglutinoid, but the latter has greater affinity for the bacteria, no agglutination may occur until the serum is so far diluted that there is only an infinitesimal amount of the agglutinoid left in the volume used for experiment.

There is some evidence that although agglutination and bacteriolysis are separate processes, yet bacilli are rendered less virulent by agglutination. Besredka<sup>1</sup> found this to be the case with typhoid bacilli, animals being able to withstand larger doses of the clumped organisms than of the normal variety. Bright and Temple<sup>2</sup> had previously noted that the bacilli are maimed, but not killed, by agglutination.

It is held by Ruffer and Crendiropoulo<sup>3</sup> that the agglutinating substances are formed by the leucocytes, especially the multinucleate variety generally associated with inflammation.

Both agglutinins and bacteriolysins "fall out" with one of the globulin fractions when serum is precipitated with ammonium sulphate. Thus the typhoid agglutinin falls out with the euglobulin in the serum of goats and rabbits, but with the pseudo-globulin in the serum of the horse. Cholera agglutinin is precipitated with the euglobulin in the serum of both horse and goat.<sup>4</sup>

**Group-reactions.**—As was noted previously in the case of the precipitins, so also with the agglutinins, the

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1901, xv. 207.

<sup>2</sup> *Brit. Med. Journ.*, 1897, i. 206.

<sup>3</sup> *Ibid.*, 1902, i. 821.

<sup>4</sup> Pick, *Hofm. Beitr.*, 1901, p. 384.

reaction brought about is not absolutely specific. A precipitating serum may react not only with human albumins, for example, but also with those derived from apes, a nearly allied zoological order; and a serum which agglutinates, for example *B. typhosus*, may also cause clumping with a culture of *B. coli*, a closely related form. In both cases, however, it is found that the serum acts in much greater dilutions upon the particular protein or bacterium respectively used for its preparation, than upon the allied forms. Thus a serum prepared by inoculation of *B. typhosus* may agglutinate this organism when diluted 1 : 1,000, whereas it may agglutinate *B. coli* only in a dilution of 1 : 50 or less, and *B. paratyphi* in dilutions of 1 : 25 or under. This phenomenon is supposed to be due to the fact that the bioplasm of different species, while consisting mainly of the same substances, contains different proportions of these constituents. Nearly allied species will contain considerable quantities of the same protein, though in one it may be largely predominant, in others relatively less in amount. Hence arise varying degrees of reaction to a serum containing an antibody to this particular protein.

**Opsonins.**—French observers, studying the phenomena of phagocytosis under the influence of Metchnikoff, early discovered that the activity of the leucocytes was greater in the presence of serum than without it (Denys and Leclef). This was confirmed by Mennes and others. The influence of the serum has been specially studied by Wright and his collaborators, the name “opsonins” having been coined for the chemical substances at work (Wright and Douglas).<sup>1</sup> Increase in rapidity of phagocytosis might theoretically be brought about either by an increase in the voracity of the leucocytes or by a diminution in the resistance offered by the bacteria to their attack; both processes have been supposed to occur. The existence of substances which increase the activity of the leucocytes (“*stimulins*,” Metchnikoff, Leishman) is now doubted by most observers, the action of

<sup>1</sup> *Proc. R. Soc. Lond.*, 1903, lxxii. 357; 1904, lxxiii. 128.

opsonins being to render the bacteria either more attractive (chemiotactic) or less resistant to the phagocytic cells. Some doubt has existed as to the possibility of phagocytosis in the absence of serum, but it seems to be established that it does occur, though only to a slight degree. The presence of normal serum increases the activity of the leucocytes in ingesting all kinds of bacteria; and when infection with a pathogenic organism has occurred and been successfully resisted, the opsonic power of the serum is found to be increased towards that particular organism. It appears that opsonins formed in response to the stimulus of an infective organism are strictly specific. There seems reason to hold that the opsonin of normal serum is distinct from those of "immune" serum, inasmuch as the former is destroyed by heating the serum to 65° C. (thermolabile) and is active against many or all kinds of bacteria, while the latter are thermostable and definitely specific. The opsonic power of a serum rapidly diminishes when it is kept at room-temperature, Knorr<sup>1</sup> finding that it is reduced by one-half in twenty-four hours and entirely abolished in five days.

The exact nature of opsonins and their relation to other substances concerned in immunity are doubtful. Greig Smith<sup>2</sup> holds that they are identical with agglutinins. Verney<sup>3</sup> believes that all varieties of immune substances—complements, copulas, antitoxins, etc.—can act as opsonins. Seeing that in a mixture consisting of serum, leucocytes, and virulent and saprophytic bacteria the leucocytes ingest the saprophytes much more readily than the parasites, it is reasonable to suggest that the opsonin acts by neutralizing the toxin or "aggressin" (Bail) by which the pathogenic germ repels the phagocyte. Grüber and Futaki<sup>4</sup> regard them as complements, owing to the destruction of normal

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1907, xlviii., No. 15.

<sup>2</sup> *Proc. Linnean Soc. N.S.W.*, 1905.

<sup>3</sup> *Policlin.*, 1907, Sez. Pract., No. 40.

<sup>4</sup> *Münch. med. Woch.*, 1906, liii. 249.

opsonins by heat (and their rapid disappearance when the serum is kept is another point of resemblance). The converse of this argument is used to support the view that "immune" opsonins are really copulas (ambocaptors), since they resist a temperature of 60° C. But the question is at present unsettled, although the bulk of the evidence appears to favour the last-mentioned hypothesis. Some chemical substances, such as formalin, chloroform, and alcohol, act as "anti-opsonins," diminishing phagocytic activity (Hektoen). Anti-opsonins are also said to be formed in cultures of bacteria (Tchistovitch and Jurievitch):<sup>1</sup> these latter are probably toxins or aggressins. The existence in opsonins of two groups, analogous to the haptophore and toxophore groups of a toxin (p. 25), is maintained by Hektoen<sup>2</sup> and Kurt Meyer.<sup>3</sup>

**Anaphylaxis.**—When the serum of one species of animal is injected into an individual of another species, antibodies are formed as has already been described. But in addition to this formation of antibodies, or as a part of the process, changes of a nature which is not understood take place in the injected animal; by these it is rendered peculiarly sensitive to a further injection of the same kind of serum. Symptoms of severe constitutional disturbance, and sometimes even death, may ensue upon such a second injection. Thus, if a guineapig receives an intraperitoneal injection of horse-serum, it suffers no ill effects; but if the injection is repeated in about twelve days' time, it becomes seriously ill, with symptoms of collapse, vomiting, bloody urine and fæces, dyspnœa, and convulsions; and may die rapidly or almost instantaneously (anaphylactic shock). The phenomenon is called "anaphylaxis" (supersensibility or hypersensibility). This condition does not develop immediately, but only after the lapse of a definite in-

<sup>1</sup> *Rousski Vrach*, 1908, vii. 669.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, 1906, xli., No. 19.

<sup>3</sup> *Berlin. klin. Woch.*, 1908, p. 951.

terval varying with the serum employed and the animal inoculated. It may pass off gradually in a month or more, or may persist for an indefinite length of time. If in the interval between the first injection and the appearance of anaphylaxis another injection of the serum in question be given, protection against the subsequent development of anaphylaxis is produced (*anti-anaphylaxis*, Richet)—a phenomenon which has been taken advantage of by Besredka in the treatment of diseases by therapeutic serums (*see* p. 47). Apparently the increased susceptibility is connected in some way with the formation of protective antibodies, of which Richet considers it to constitute the first stage. Milk, egg-albumin, bacterial proteins, and other substances are all capable of inducing anaphylaxis.<sup>1</sup> The condition is of interest as an explanation of many cases in which ill effects have followed the use of antitoxic serum (*see* p. 55). Besredka<sup>2</sup> finds that heating the horse-serum deprives it of toxic power, and that if the animal is anæsthetized at the time of the second injection, no ill effects result.

**Protective ferments.**—Very closely related, in all probability, to the phenomena of anaphylaxis is the discovery of Abderhalden that the presence of minute amounts of any foreign or unusual protein in the blood-stream of a living animal gives rise to the formation of a specific ferment capable of breaking up this particular protein into simpler bodies resembling the products of digestion. This property of ferment-formation is active not only against proteins derived from other species of animals, but also against the products of the cells of the animal's own economy. So that in pregnancy ferments are formed capable of breaking down placental tissue, small quantities of which enter the maternal circulation; and in conditions

<sup>1</sup> Rosenau and Anderson, *Bull. Pub. Health and Mar. Hosp. Serv. U.S.A.*, 1907, No. 30; 1906, No. 29; and 1909, No. 50.

<sup>2</sup> *Compt. Rend. Soc. Biol.*, 1907, lxii. 1053. *See* Richet, *Ann. de l'Inst. Pasteur*, 1907, xxi. 497.

of disease, when the cells of certain tissues undergo destruction, ferments are recognizable which act only on the proteins of these particular cells. Possibly similar ferments are formed against foreign carbohydrates and fats, but this does not appear to be so certain. This peculiar property of forming special ferments capable of destroying (catalysing) foreign proteins appears to be protective in character, the cells of the tissues being thus saved from any prolonged contact with abnormal constituents of the serum; these are broken down into those simpler "cleavage products" which are common to all proteins and are suitable for use by the cells as building materials to be recombined in their own protoplasm.

**Ehrlich's theory of immunity.**—Having thus briefly sketched the peculiar properties which are conferred on the blood-serum by contact with bacteria and other bodies, it remains to consider the theory of the production of immunity and allied phenomena which at present holds the field. This is due to Ehrlich, and is known as his "side-chain" hypothesis. The name is taken from organic chemistry, in which complex molecules have the property of picking up and combining with other atom-groups. Thus in the example given in Fig. 5 we see that a benzene nucleus has joined to itself three  $\text{NO}_2$  groups and one OH group, becoming trinitro-benzene or picric acid.

The chemical processes which occur in living protoplasm are, of course, much more complicated than those of inorganic matter. Instead of a comparatively simple change brought about once and for all, as in the interaction of two simple salts, or the rather more complex phenomena of organic chemistry, we have a continual series of changes taking place between a mass of protoplasm and the surrounding lymph. The molecule of living matter is itself vastly complex. We know that it can break down into a number of simpler substances, such as albumin, globulin, polypeptides, lecithin, etc., each of which is in reality a complex body, yet all of which are loosely or tightly bound



together into a huge molecule of protoplasm. Of the true nature of this last we have no real knowledge. For the purpose, however, of forming a mental picture of the chemical processes taking place in living matter, we may imagine the cell as consisting of a central mass—corresponding with the ring of a benzene molecule—to which are united outlying groups of molecules that have the power of entering into combination with other substances circulating in the lymph, such as particles of food, etc. These outlying groups are the “side-chains” of Ehrlich’s theory. Thus a side-chain attached to a cell

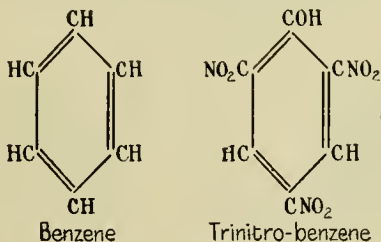


Fig. 5.

may join to itself a particle of oxygen, of carbohydrate, of fat, etc., and thereby take part in the nourishment of the cell; or it may become united with a molecule of poison, such as the toxin of the diphtheria bacillus. In the latter case, either of two things may conceivably happen: the toxin may, through the medium of the side-chain, become part of the whole cell and may thus poison it, producing actual death (necrosis) or degeneration (e.g. cloudy swelling); or it may cause the death only of the individual side-chain to which it has attached itself, in which case the latter is thrown off and a new one is formed by the cell. This reproductive process is supposed to represent what takes place in the presence of only a small quantity of poison, such as first reaches the cell in a case of disease.

The side-chains of living cells, in virtue of their properties of taking up food and other materials—useful and harmful—from the lymph, are known as *receptors*.

By way of illustration of the working of this hypothesis we may take the process of hæmolysis, as it affords perhaps the easiest example. Here, as we saw above (p. 10), two substances, (*a*) and (*b*), are necessary to effect destruction of the corpuscle. Ehrlich's theory supposes that the protoplasm of the corpuscle has not the power of combining directly with the dissolving substance (complement) which is always present in serum, but that it can attach to itself a second body (copula), produced in immunized animals, which

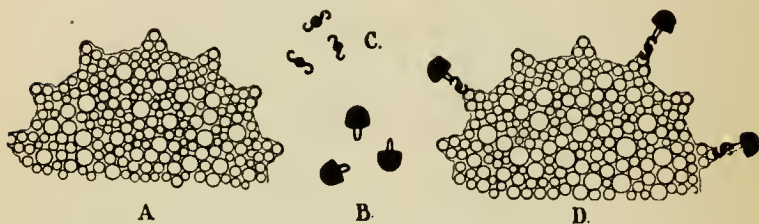


Fig. 6.—Diagrammatic representation of hæmolysis (or cytotoxicity). A, Corpuscle or cell, consisting of molecules of different sizes, with special groups (side-chains, receptors) at the periphery. B, Molecules of complement. C, Copula or immune body. D, Corpuscle with complement molecules attached to side-chains by copula.

in its turn can grapple to itself the solvent ; and that thus the destructive matter is enabled to combine with the corpuscle and dissolve it. This process is illustrated in Fig. 6.

An exactly similar process is at work in bacteriolysis ; the ferment present in the serum being attached to the bacteria by a copula, immune body, or bacteriolysin, which is itself a side-chain thrown off from some of the tissue-cells.

The process by which a bacterial toxin acts on a cell, though at first sight more direct, is found to be very similar to the action of a hæmolytic substance on a corpuscle ; only the toxin consists of both destructive substance



and uniting substance joined together in one molecule. The two parts in this case are called respectively the toxophore and the haptophore (τοξικόν, poison; φέρω, I carry: and ἄπτω, I join; φέρω, I carry), the latter corresponding to the copula. The combined toxic molecule seizes on an appropriate side-chain of a cell; and if a number of side-chains thus take up poisonous groups, the cell itself dies. If only one or two side-chains are thus attacked, they are themselves killed and drop off, but the cell escapes. The uninjured portion of the bioplasm then proceeds to put out a fresh supply of the particular kind of side-chains, of which some have been killed. As frequently happens in living bodies, the repair goes beyond the original supply (according to Weigert's "Overproduction Theory"), and the cell thus becomes furnished with an increasing number of the side-chains capable of fixing the particular toxin.

But as this process goes on the cell forms so many side-chains that it cannot keep them all attached to itself, and some of them are cast off into the lymph around the cell and ultimately get into the blood. *These free side-chains constitute the antitoxin.* They are capable of uniting with the molecules of the toxin before it reaches the cells, and in this way they prevent any poisonous action resulting. Further, if the serum containing these free side-chains is injected into another animal, they will still perform the same office under their new conditions, and will confer on the second animal the same immunity as was possessed by the original immunized one. The curative and prophylactic action of antitoxin is thus explained (*see* Fig. 7). If we add to some toxin an equivalent quantity of antitoxin,<sup>1</sup> the molecules of poison present

<sup>1</sup> Properly speaking, antitoxin is the actual substance which combines with the toxin and neutralizes it. In ordinary parlance the word is used for the serum containing the antitoxic body. For further consideration of the interaction of toxin and antitoxin, *see* p. 101.

become combined with the free side-chains in the antitoxin, and can no longer attack the tissue-cells. Hence the injection of a mixture of the two is innocuous. If the bacteria have already gained a footing in the patient and are pouring out constantly a stream of toxin, the injection of a dose of antitoxin neutralizes the poison; but it is necessary to give a very large dose of it in order to meet the continuous inflow of toxin. If, on the other hand, a person has not yet got, say, diphtheria, but is exposed to the chance of infection, then a protective dose of antitoxin

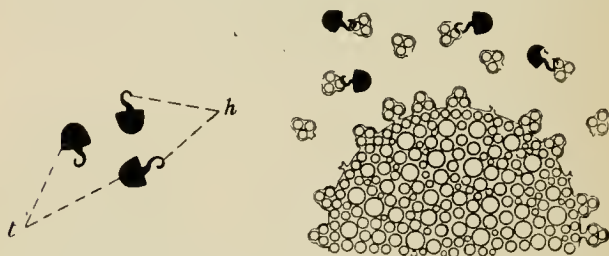


Fig. 7.—Diagram of cell with numerous side-chains (receptors) produced by stimulation with toxin. Some of these have been cast off as antitoxin, and are combining with molecules of toxin. On the left are seen molecules of toxin, showing *t*, toxophore, and *h*, haptophore, elements.

may be given to anticipate infection—so as to be lying in wait, as it were, for any poison that may be formed.

To this last application it might be objected that, as previously stated, the antitoxin is not antibacterial, and that therefore it does nothing to stop infection. But as the bacilli conduct their conflict with the body by means of their poisons, injuring the cells, and so preventing them from forming antibacterial matter, the neutralization of the first doses of poison enables the organism (the animal attacked) to gain time to form its defensive weapons. To use a military simile, we may do much to resist the first assault if we can damp the powder of our antagonists, and so we may enable our own reinforcements to come into action.

It is important to remember that by administering antitoxin we only neutralize the *free* toxin present. That which has already entered into combination with the side-chains of the tissue-cells is practically beyond the reach of the remedy. It is possible that, if there is a very large quantity of antitoxin present, some kind of "mass" action may take place, whereby the toxin may be withdrawn from its combination with the cells and caused to combine with the antitoxin instead; but this is not absolutely certain. Hence arises the urgent need, in giving antitoxin, to give it as early as possible in the disease. For example, in the case of tetanus, it may be too late to make use of antitoxic serum when the symptoms of the disease have appeared, the poison being already closely attached to the cells; hence many failures and much disappointment in the treatment of this disease.

It may be pointed out that so long as the side-chains are attached to the cells they are a source of weakness, as enabling the toxins to attack them, whereas, when they are cast off into the serum as antitoxin, they become a protection. A toxin can only act if it finds appropriate receptors to which it can attach itself; otherwise it would circulate harmlessly in the lymph. Since it would be better for the cell, from the point of view merely of its relation with toxins, if it had no side-chains, it is supposed that these exist originally for some other purpose; and this is considered to be the assimilation of nutritive materials, as already suggested. These presumably are taken up by the cell, in the same manner that toxins are attached, by means of the affinities possessed by the side-chains.

A distinction is held to exist between the toxins formed by bacteria on the one hand—and with them must be grouped the poisons elaborated by poisonous snakes, and those resident in certain plants, such as ricin, abrin, etc.—and the ordinary mineral and vegetable poisons—mercury, arsenic, strychnine, morphine, and the like. It is supposed that the substances which form the first group are proteid

in character (globulins, etc.), and that it is towards them especially that the side-chain activity of the cell is directed. They are capable of acting as antigens (p. 12). The poisons of the second group appear to act differently, as in the case of these substances no antitoxic serum can be prepared. The cell as a whole is either killed or recovers from the effects of the poison, but it cannot protect itself by throwing off side-chains to neutralize the poison. This property is distinct from the power which the body undoubtedly possesses of accustoming itself to increasing doses of a poison such as morphine or arsenic. Nevertheless, even in the case of such a poison as arsenic, absorption by means of receptors has been supposed to take place, and Ehrlich believes that certain chemical groups, such as acetyl, have the property of acting as copulas and attaching the poison to the living cell, in virtue of the affinity between these and the organic radical in question. (*See also* Chapter xxi., p. 412, *Chemotherapy*.)

**Incubation period.**—A peculiar feature in the action of bacterial toxins is the occurrence of an incubation period between the administration of the dose and the onset of symptoms. Thus, by giving increasing doses of tetanus toxin, the rapidity of the onset of spasm may be increased up to a certain point; but after this is reached, no further addition of poison will accelerate the event. It may be suggested that time is needed, not only for the combination of the toxin with the side-chains—which is probably a somewhat slow process—but also for the absorption of the poisoned receptor into the general body of the cell, which must precede symptoms of intoxication.

Before leaving this subject it may be well to emphasize the fact that the explanation given by Ehrlich of the phenomena of bacteriolysis, the action of toxins, etc., is pure hypothesis. The hypothesis has been fruitful, suggesting new lines of research; and so far the results obtained are mainly consistent with the theory. But care must be taken not to confuse the fascinating diagrams by which

we are enabled to form a mental picture of the events that take place, with realities. An antitoxin is probably analogous to a secretion, and the process of bacteriolysis is a chemical reaction into which three bodies enter—very similar to the interaction of the fibrin ferment, fibrinogen, and calcium salts to form actual fibrin. In the present account the illustrations have been kept as diagrammatic as possible, at the expense of verisimilitude and artistic merit, in order to avoid any undue pretence to reality.

We have thus seen that the serum of an “immunized” animal may contain not only antitoxic substances capable of neutralizing the poisons of bacteria, and antibacterial bodies (*complement* and *copula*) fitted to destroy the organisms, but also substances which sensitize bacteria (*opsonins*) or which agglutinate bacteria or corpuscles (*agglutinins*), others which destroy living cells (*cytolysins*), and others still which cause a precipitate with the albumins of the serum of other species (*precipitins*).

**Forms of immunity.**—It remains to explain, on the theories just set forth, the various forms of immunity already alluded to. In the case of species, races, or individuals who are *naturally* immune to certain infections, we must suppose either that they possess no side-chains capable of uniting with the toxins of the bacterium causing the disease, the latter thus becoming harmless,<sup>1</sup> or that they normally contain in their systems the two substances necessary to repel the bacteria, viz. the complement and the copula. A further possibility may be considered, arising from the peculiar quality of poisons by virtue of which they injuriously affect only certain tissues. Thus,

<sup>1</sup> An instance of this condition may be seen in the tortoise, which is immune to the toxins of tetanus; if, however, the blood of a tortoise which has received a dose of tetanus toxin be injected into a susceptible animal, it will cause death, showing that the poison is not neutralized in any way, but merely has no power of affecting the cells of the tortoise.

morphine acts on the cells of the brain, phosphorus on the liver, diphtherial toxin on the cardiac muscle and peripheral nerves, tetanus chiefly on the spinal cord. If in any individual there are cells of other tissues which have greater affinity for a toxin than have those cells which it possesses the power of injuring, the former may absorb the poison—with impunity—and leave none to cause symptoms of poisoning. In those who are *artificially* immunized or who have recovered from a disease (*acquired immunity*) a bacteriolytic copula has been produced, as has been described (along with opsonins, agglutinins, etc.), by gradual education of the cells to throw it off, or a power of forming antitoxin has been similarly acquired. In all these cases the immunity is said to be “active.” When an animal has received into its system a dose of antibacterial or antitoxic serum and is thereby enabled to resist a disease, it is said to possess “passive” immunity. This lasts only as long as the injected serum remains in the body; and this is not, as a rule, for any long period of time, since the foreign serum is more or less rapidly excreted.<sup>1</sup> The animal in this latter case has not gained for itself any power of forming protective substances; whereas in active immunity its cells have been educated to perform this duty, and this acquirement seems to be retained either permanently or for a considerable period of time. It is found that, if an individual has gained this active immunity to one disease and then becomes infected with a second distinct malady, the former protective power is often lost.

Welsh has suggested that in cases of infection a conflict may be supposed to occur between a bacterium and the body-cells, each side replying to the destructive substances brought against it by its opponent, with antibodies capable of neutralizing them—toxin being met by anti-

<sup>1</sup> The antitoxin or copula injected may perhaps be neutralized by the formation of an anti-antitoxin or anti-copula, and not merely passed out of the body; or it may be broken up by a special ferment, as are other foreign proteins (p. 21).



toxin, bacteriolysin by antibacteriolysin, and so forth. Enough has been said, at any rate, to show the immense complexity of the serum, and the capacity possessed by animal bodies of protecting themselves in many ways against injurious influences.

**Modification of phenomena of bacteriolysis, etc., in the presence of living tissues.**—It might seem from a consideration of the foregoing facts that the explanation of immunity was fairly simple, but other ascertained facts render the question more complicated. Thus, taking the case of anthrax bacilli, we find that the blood of the rabbit, a highly susceptible animal, acts destructively upon the organisms in a test-tube : within the body it manifestly does not do so. Similarly, all white rats possess serum which destroys anthrax bacilli, but they are not all immune. On the other hand, the serum of animals immune to the disease, as that of the hen, forms a good culture-medium for the bacillus. It thus becomes clear that we have to take into account not only the blood, but also the living tissues by which it is surrounded in the body. In the one case the tissues seem to exercise some inhibitory influence over the bacteriolytic action of the blood ; in the other, they supply some factor necessary for the defence of the animal against the bacilli. Further experiments show that while the serum of the rabbit is destructive for anthrax bacilli, an extract of its organs prepared by triturating them with salt solution has no such power. Indeed, if crushed organs are added to the serum and the bacilli exposed to the action of the mixture, no bacteriolysis takes place. The tissue-cells have in some way deprived the serum of its bacteriolytic property.

The explanation<sup>1</sup> that is given of these phenomena is as follows : We have already seen that a copula, or immune body, is needed to fasten the ferment to the bacteria and so produce their destruction. This copula has affinity for the tissue-cells—in this case an even greater affinity than it has

<sup>1</sup> Bail, *Prager med. Woch.*, Nov. 25, 1903, p. 307.

for the bacilli—hence it unites with the cells, and is no longer available for the process of bacteriolysis.

**Maternal transmission of immunity.**—The question of the transmission of immunity from mother to offspring is one of considerable interest, but is not yet satisfactorily elucidated. Agglutinating power is not usually transmitted in the case of enteric fever, nor in tuberculosis; but occasionally such transference is found.<sup>1</sup> Hæmolysins are transmissible in animals (Kreidl and Mandl),<sup>2</sup> and immunity to diphtherial toxin (six out of twelve guinea-pigs, Anderson):<sup>3</sup> so too is supersensibility to horse-serum, according to this last observer. Immune bodies of different kinds may pass into the milk: thus Courmont and Cade<sup>4</sup> found that agglutinins were transferred to an infant through the milk of a wet-nurse, and Salge<sup>5</sup> finds that diphtherial antitoxin may be so transmitted and absorbed by the infant. According to v. Eisler and Solhma,<sup>6</sup> opsonins are not transmitted by the mother to the fœtus in utero, but they are present in the milk. In all these cases the properties conferred are merely passive and are soon lost by the offspring. The father, as might be expected, has no power of transmission of immunity (Remlinger).<sup>7</sup>

**Absorption of toxins and antitoxins by the mouth.**—Closely allied to the question of maternal transmission of immunity is that of the absorption of antibodies when administered by the mouth. Hewlett<sup>8</sup> found that diphtherial antitoxin administered by the mouth was

<sup>1</sup> Rodhain, *Prager med. Woch.*, 1903, iii., Hft. 3.

<sup>2</sup> *Wien. klin. Woch.*, 1904, No. 22, p. 611.

<sup>3</sup> *Bull. Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Serv.*, 1906, No. 30.

<sup>4</sup> *Compt. Rend. Soc. Biol.*, Nov. 25, 1899.

<sup>5</sup> *Jahrb. f. Kinderheilk.*, 1904, lx. 1.

<sup>6</sup> *Wien. klin. Woch.*, 1908, No. 19.

<sup>7</sup> *Ann. de l'Inst. Pasteur*, 1899, xiii. 189. See also Merkel, *Münch. med. Woch.*, Feb. 23, 1904, p. 329; Covazza, *Il Policlin.*, Mar. 5, 1903; Bertarelli, *Centralbl. f. Bakt.*, 1906, Orig., xli. 767.

<sup>8</sup> *Lancet*, 1902, i. 375 (*Proc. Path. Soc. Lond.*).



nseless in protecting animals against the poison of the disease, and this is confirmed by Salge,<sup>1</sup> who finds, however, that any antitoxin present in the mother's milk is absorbed. If this is confirmed, it opens up an interesting question in proteid digestion; for it is usually held that proteins are broken up into their constituent amino-acids and other bodies in the process of digestion and re-synthesized in the system. Diphtherial antitoxin is probably a complex proteid body and suffers the same fate; but it would seem that the proteins in mother's milk, being closely related to or identical with those of the child, may be absorbed intact. Toxins given by the mouth (e.g. tuberculin) are said by some observers to be absorbed, but according to Ransom<sup>2</sup> and to Tshitkine<sup>3</sup> the toxins of diphtheria and tetanus do not enter the system by this route. Further experiments are, however, necessary in regard both to this and to other toxins, as McClintock and King<sup>4</sup> state that in rabbits some absorption of antitoxin occurs from the alimentary canal, and that this may also occur in man; while Onorato<sup>5</sup> states that similar absorption takes place in the guineapig, the antitoxin being recognizable in the blood within twelve hours, and present for over a fortnight. Vaccines (i.e. dead bacilli) given by the mouth are said by some observers to exert the same immunizing effect as when administered subcutaneously; a suggestion vigorously contradicted by others. Here again further evidence is needed.<sup>6</sup>

**Source of antibodies.**—With regard to the site of production of the complement or alexine, there is some evidence that the leucocytes constitute at least one source

<sup>1</sup> *Op. supra cit.*

<sup>2</sup> *Deut. med. Woch.*, 1898, p. 117.

<sup>3</sup> *Ann. de l'Inst. Pasteur*, 1905, xix. 335.

<sup>4</sup> *Journ. Infect. Dis.*, 1906, iii. 701.

<sup>5</sup> *Ann. Ist. Maragliano*, Genova, 1904, i. 159.

<sup>6</sup> See Loeffler, "Immunisierung per Os," Berlin, 1906. Wright, *Lancet*, 1908, ii. 232. Also *infra*, p. 117.

of such bodies. Thus, dog's serum alone is found not to act bacteriolytically on anthrax bacilli, but if some leucocytes from the same animal are added to the serum, then destruction of the bacilli takes place. From other data it is believed that the serum of dogs contains the immune body, so that it appears that in this case the leucocytes are the source of the complement (Briscoe).<sup>1</sup> This view, corresponding with that of Metchnikoff, has recently been disputed owing to the fact that leucocytes seem to have the power of absorbing complement from fluids containing it (Hoke).<sup>2</sup> In other cases it has been possible to supply the complement by the addition of an extract of the tissues, so that these also must be regarded as forming ferments capable of acting bacteriolytically in the presence of a suitable copula.

Complement is not normally present in cerebro-spinal fluid, nor is there any in oedema fluid or in purulent exudates, but it is found in inflammatory exudates which are not purulent, in the fluid of blisters, and so forth (Muttermilch and Hertz).<sup>3</sup> Gurd<sup>4</sup> states that there is no free complement in plasma, but only a forerunner or "complementogen."

Of the chemical nature of complement nothing is known; indeed, no antibody has ever been isolated and identified. Complement is usually regarded as being allied to the ferments or enzymes—an equally unknown chemical group. Ferrata<sup>5</sup> found that it was separable into two parts, one of which fell out with the globulin of the serum (*Mittelstück*), while the other remained in solution with the albumin (*Endstück*). Neither part alone is active, but on mixture

<sup>1</sup> Prof. Orth's "Festschrift" (Berlin, 1903), p. 396 *et seq.*; see *Brit. Med. Journ. Epit.*, June 27, 1903, p. 104.

<sup>2</sup> Quoted by Kaplan, "Serology of Nervous and Mental Diseases," 1914.

<sup>3</sup> *Zeitschr. f. klin. Med.*, 1912, p. 404.

<sup>4</sup> *Journ. Infect. Dis.*, 1914, xi. 142.

<sup>5</sup> *Berl. klin. Woch.* 1907, p. 366.

of the two the power of acting as complement is regained, though not to the original extent.

It is generally held that other antibodies are formed in the hæmopoietic system—the spleen, lymphatic glands, bone-marrow—or even in the blood-stream itself.<sup>1</sup>

Copula may possibly be derived from leucocytes, as Eyre<sup>2</sup> found a general correspondence between opsonin formation and leucocytosis in pneumonia. Allen has adduced some evidence that thermostable antibodies are formed in the muscle-cells. Hektoen and Carlson<sup>3</sup> found that toxin rapidly disappeared from the circulating blood of an animal, and that this blood was not capable of giving rise to formation of antitoxin if injected into another animal. But if the first animal which had received the toxin was bled and transfused with the blood of a normal animal, the formation of antitoxin still went on, showing that the toxin had been fixed by the tissues and that the formation of antitoxin continued in them.

The quantity of antibodies present in the serum is said to be indicated by the amount of globulin precipitated on dropping dilute acetic acid into diluted serum or blood (Rivalta's reaction); it is also stated to correspond with the degree of leucocytosis present in infective conditions (Gironi).<sup>4</sup>

The formation of antibodies is stimulated by the occurrence of hæmorrhage or by fever; also by injection of small doses of mercuric chloride, which acts as a protoplasmic poison (Kalledey).<sup>5</sup>

**Plurality of complements.**—Considerable controversy has centred round the question whether the com-

<sup>1</sup> For literature see Reiter, *Zeitschr. f. Immunitätsforsch.*, 1913, Orig., xviii. 5.

<sup>2</sup> *Lancet*, 1908, i. 539.

<sup>3</sup> *Journ. Infect. Dis.*, 1910, vii. 319.

<sup>4</sup> *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1912, xxv. 229. Cf. Eyre, *op. supra cit.*

<sup>5</sup> *Centralbl. f. Bakt.*, lxviii. 358.

plements or alexines, by which, for example, bacteriolysis is brought about, are the same for all micro-organisms, and the same in all species of animals. Thus, it might be possible to prepare an immune serum (one containing a copula or intermediary body) which should be capable of immunizing a certain species of animal (A) against a particular bacillus, but this intermediary body might only be capable of uniting to the bacilli a special form of complement such as exists in the kind of animal (A) used. We cannot be certain without making the actual experiment that it will act in the same manner within the body of a second species of animal (B): it may be incapable of uniting with the form of complement which is here present. For example, we might prepare an antityphoid serum capable of protecting an animal (say, horse) against typhoid bacilli, i.e. of causing destruction of *B. typhosus* when it is injected into this animal; but it does not necessarily follow that it will act as a cure in cases of enteric fever in man, since human beings may not possess the kind of complement with which horse-copula can unite so as to attack the micro-organisms.

It has also been a moot point whether in a single species of animal there is present only one complement, which is ready to act upon all bacteria alike, and upon blood-corpuscles, cells, etc., provided it is supplied with the requisite intermediary body; or whether more than one complement is present in the serum—one perhaps capable of producing hæmolysis, another of causing bacteriolysis. The mode of experimenting is by “preparing” or “sensitizing” (p. 11) bacteria and corpuscles with a certain immune body, and then adding these prepared bacteria or corpuscles to a specimen of serum till no more lysis takes place. When the serum has thus been saturated with one kind of organism or corpuscles till it can dissolve no more, a second variety of prepared body (corpuscle or bacteria) is added, and it is seen whether destruction of any of this occurs. Results appear to be contradictory. Bordet and

Büchner hold to the unity of the complement; Metchnikoff, Neisser, Ehrlich, and Morgenroth support a plurality.<sup>1</sup> It is quite possible that the same answer to the question may not hold good in all species of animals. There is considerable evidence, however, pointing to the fact that—at all events, in some instances—the complement which causes hæmolysis may be different from that causing bacteriolysis in the same animal. Neisser<sup>2</sup> has shown that rabbit's serum can be deprived of its bacteriolytic complement by addition of anthrax bacilli, but that it still remains capable of hæmolysis. Gengou and Tarassevitch<sup>3</sup> have adduced experiments tending to show that different kinds of leucocytes are the sources of hæmolytic and bacteriolytic complements respectively—the “microphages” producing complement necessary for bacteriolysis, the “macrophages” that for hæmolysis.

Forster<sup>4</sup> finds that the same complement in goat's serum acts against the organisms both of cholera and of typhoid.

**Dangers of excess of antibodies.**—It has been suggested<sup>5</sup> that the presence of excess of antitoxic serum may have an ill effect, since the antitoxin which is not employed in neutralizing toxin might give rise to the formation of anti-antitoxin, which would prevent the action of antitoxin in the future stages of the disease. This does not appear, however, to constitute a practical danger in therapeutics; and considering the entire ignorance which exists with regard to the exact quantity of toxin present in any given case, we must continue to be guided by purely empirical rules for administration of antitoxins. A danger similar to that just mentioned is said to exist in

<sup>1</sup> See *Ann. de l'Inst. Pasteur*, 1899, 1900; *Berlin. klin. Woch.*, 1899, 1900, 1901.

<sup>2</sup> *Deut. med. Woch.*, 1900.

<sup>3</sup> *Ann. de l'Inst. Pasteur*, 1900, 1901.

<sup>4</sup> *Lancet*, 1905, ii. 1531.

<sup>5</sup> See Ainley Walker, *Clin. Journ.*, June 17, 1903, p. 144.

the case of antibacterial serums. If an excess of such a serum be administered, there is produced an excess of copula in the absence of sufficient complement. When the former unites with the bacteria, its affinity for the complement appears to be reduced; at all events, it is not increased. The free copula (the excess) then appears to attach itself to the available complement, and we have bacteria with copula attached to them, and complement molecules with copula attached to them. This double combination seems to prevent bacteriolysis, as it would be necessary, in order that this should occur, for copula to unite with copula, which is not possible.

**Deficiency of complement.**—So far we have paid more attention perhaps to the copula than to the complement in the production of immunity. But susceptibility to disease may depend on lack of sufficient complement as much as on defect of the intermediary. Some individuals may be naturally ill supplied with complement. In others pre-existing disease may exhaust the supply. Thus it has been shown that in chronic maladies (carcinoma, Bright's disease, etc.) the quantity of complement present in the serum tends to fall, and in this way we may explain the tendency to terminal acute infections in these conditions. Excessive exertion may perhaps cause destruction of complement, and thus predispose to infectious diseases. The use of such remedies as yeast and cinnamic acid may lie in their power of supplying complements, the former perhaps directly, the latter by stimulating leucocytosis—the leucocytes being, according to some authors, the main source of complement.

**Fixation or deviation of complement.**—As already mentioned (p. 8), if a hæmolytic serum be heated, so as to destroy the complement present (“inactivated”), and be then brought into contact with appropriate blood-corpuscles, the copula present attaches itself to these (Fig. 8, A), and hæmolysis will result if a supply of complement is provided by contact with fresh serum. (It is found that the complement may in many instances be provided by the serum

of quite a different species of animal : thus, with a mixture of heated hæmolytic rabbit's serum and corresponding ox-corpuscles, hæmolysis will result on addition of goat's serum.) If, on the other hand, the complement and the copula be left in contact with one another (Fig. 8, B), no union results : consequently, in such a mixture as the latter (B) the complement is still free, and will bring about hæmolysis if the second mixture is added to the first (A), in which there is copula along with corpuscles. If, however, to the second mixture (B = complement + copula) some appropriate

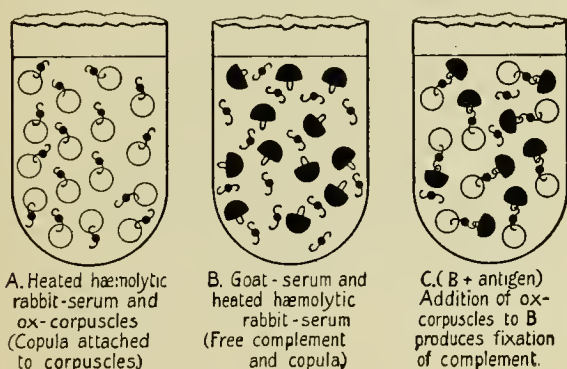


Fig. 8 (*see text*).

corpuscles be added, hæmolysis ensues (Fig. 8, c): the complement is used up in the process and is no longer free to produce further hæmolysis in mixture A. A means of testing for the presence of the appropriate corpuscles is thus provided ; for if a liquid containing appropriate corpuscles is added to mixture B, the complement in the latter is fixed (attached by the copula to the corpuscles) and hæmolysis occurs ; whereas if the corpuscles added are inappropriate (belonging to some other species of animal than that used for the original preparation of the hæmolytic serum, and therefore unable to become attached to the copula), no fixation occurs and the complement remains free—



consequently no hæmolysis takes place. Not only blood-corpuscles but many other albuminous bodies, when injected into animals of a different species, produce corresponding antibodies (copulas). Each of these, by union with the existing complement, is able to combine with its appropriate antigen—thus the test may be applied to detect the presence of any specific antibody.

For example, if a mixture be made of (*a*) syphilitic antigen, represented by an extract of syphilitic fetal liver, (*b*) syphilitic antibody contained in inactivated serum from a case of syphilis, and (*c*) complement, these three bodies become linked together, and the addition of the mixture to sensitized red corpuscles (as, for instance, in mixture A, Fig. 8) is not followed by hæmolysis, as the complement is “fixed” to the syphilitic “antigen-antibody” combination, and is therefore not available to complete the hæmolytic system. In this connection may be mentioned the reactions associated with the names of Gengou (precipitation), Neisser and Wechsberg (bacteriolysis), and Wassermann (syphilis). *See also* Chapters IV. and XXI.



## CHAPTER II

### PREPARATION AND ADMINISTRATION OF SERUMS

**Theory of serum treatment.**—As previously explained (p. 4), an antitoxic serum is one which has the power of neutralizing the exotoxin or soluble poison elaborated by a particular micro-organism. It can only be relied upon to combine with free toxin encountered in the plasma or lymph of the infected animal or patient. It has no destructive effect on the organisms which are causing the disease. It may also confer a transitory passive immunity, and so exert a prophylactic action, but this immunity is rapidly lost owing to excretion of the antitoxin or to its destruction by specific ferments (p. 21). On the other hand, an antibacterial serum when fresh may contain within it the two bodies, copula and complement, necessary for effecting bacteriolysis; later it probably contains little complement and needs to be supplemented by the supply of this body present in the plasma of the patient. So far from being antitoxic, such a serum may theoretically cause liberation of an increased quantity of endotoxin by its “lytic” or solvent action on the bacteria, but it may possibly in some instances possess the power of causing further decomposition of the poisonous protein, so that ill effects are not produced. Certain serums which possess apparently some bactericidal power are yet not bacteriolytic *in vitro*. Such have been supposed to exercise an opsonizing action, and thus to increase phagocytosis.

**Preparation of antitoxic serum.**—Some account of the preparation of individual serums will be found under their separate headings; only a general outline of the

processes adopted will here be given. It is necessary, first of all, to make sure that the animal (generally a horse) selected to serve as the factory for antiserum is itself free from disease of any kind which might be transmitted to human beings. For this purpose it is submitted to a preliminary test by being injected with tuberculin (*see* p. 299) to bring into prominence any latent tuberculous infection, and with mallein (*see* p. 204) to demonstrate similarly any unsuspected glanders—the presence of either of these diseases obviously rendering it unsuitable for the production of remedial antiserum. Should the animal prove sound, it is in some cases first inoculated with a dose of attenuated (weakened) toxin, prepared by heating the virulent poison or by treating it with some chemical agent which reduces its strength. After this the animal is inoculated with increasing doses of the virulent poison at stated intervals of time. Each dose is generally sufficient to produce some febrile reaction, from which the horse recovers in the intervals. The doses are given, as a rule, subcutaneously, but they may finally be administered intravenously when a high degree of immunity has been attained. Sometimes, as a finishing touch, the bodies of the actual dead organisms are injected. The horses used for the preparation of serum generally flourish under the treatment, and grow sleek and fat. They are liable, however, after a time to develop symptoms of disease; amyloid change may occur in the liver and spleen, and a fatal hæmorrhagic hepatitis may sometimes ensue.<sup>1</sup>

The toxins used for injection are prepared by growing the organisms in suitable fluid media; the cultures are then passed through a Pasteur-Chamberland or other similar filter of porcelain, in order to remove the bodies of the bacteria. It is important that the toxins should be as potent as possible, and special methods are adopted to secure the highest possible degree of virulence.

As the site for the injections administered to the horse

<sup>1</sup> Lewis, *Journ. of Med. Research*, 1906, xv, 449.

the root of the neck is generally selected, the hair being first shaved, and the skin thoroughly scrubbed with lysol or some other antiseptic. When, after a number of serial injections, a sufficient degree of immunity has been reached, the blood is withdrawn from the jugular vein by means of a simple incision through the skin, made with all antiseptic precautions, a sterile cannula being thrust into the vein, and the blood received into sterilized vessels. In these it remains till coagulation has taken place, and the free serum is then decanted off and mixed with a small quantity of some antiseptic. It is transferred to bottles of convenient size, and is ready for use. A large quantity of blood can be obtained at a single operation from a horse (16 to 20 pints from a good-sized animal) without ill effects. It is important to wait for a few days after the last injection of toxin before withdrawing the blood, as otherwise the serum may contain poisonous material.

**Preparation of antibacterial serum.**—Antibacterial serums are produced by a method very similar to that used for preparing antitoxic serums, but the actual bodies of the bacteria are injected instead of the filtrate from a fluid medium. Sometimes the dead bodies of the organisms are first used, or an attenuated culture, the virulent bacteria being withheld till some degree of tolerance is established. A dose of corresponding antiserum may be used to mitigate the effects of the first dose, if it be available. In the case of antibacterial serums it is most important that the preparation used for treatment of disease should be freshly made, since it has been shown that the value of such serums rapidly falls.

Two special factors enter into the question of the manufacture of antibacterial serums which do not apply to antitoxic preparations. In the first place it is found that many species of bacteria comprise different strains or varieties which react differently towards protective serums. Thus, a serum may be prepared which will be fatal to a certain strain of streptococci—the variety used for its preparation

—but which will have no similar effect on another race of the same organisms, derived perhaps from a different patient or from a slightly different form of pyogenic disease. Now, in any individual case of illness we cannot tell what strain of bacteria may be present, and therefore in preparing a serum for practical therapeutics it is advisable to use several strains for the purpose of immunizing the animal, in order that the chances of combating the causal organism in any human patient may be increased. A serum thus prepared with several strains of an organism is said to be “polyvalent.”

Again, as has already been pointed out, it does not follow that the complement existing in the body of one animal will be capable of uniting with the copula supplied by another animal, so as to destroy a given bacterium. An antibacterial serum originally, no doubt, possesses in itself both complement and intermediary body. But the former is an unstable substance: it rapidly vanishes from the serum when kept,<sup>1</sup> and it is not improbable that it is destroyed when the serum is injected into another kind of animal. Hence the copula contained in the immune serum may have to depend on a complement found in the animal or man to which the serum is administered, if it is to have a bactericidal effect. If the two bodies do not fit one another, no curative result will ensue. It has been recommended, therefore, that serums for human use should be prepared from some animal nearly allied to man, such as the ape. Such a serum is said to be “homologous.”

In the case of many antibacterial serums—for example, those prepared against streptococci and pneumococci—the presence of leucocytes seems necessary to the action of the serum. Possibly it is an opsonizing influence that is exerted, rather than a true bacteriolysis.

**Testing serum.**—Before a specimen of serum is issued for use it ought to be tested, to ensure that it is free from contamination. It must not contain living bacteria or

<sup>1</sup> See Ainley Walker, *Lancet*, 1901, i. 18.

toxins. In order to test it, portions of it must be mixed with culture-medium and incubated—some aerobically, some anaerobically—to see if any bacteria develop. Some of it must also be injected into an animal to make sure that it is not toxic. Cases have occurred in which death has been caused by the use of a serum containing the toxins of tetanus.

**Standardization of serums.**—Since it has hitherto been found impossible to isolate the actual toxins of bacteria, so that no process of weighing or measuring can be applied to them, it is necessary to devise some other way of calculating their strength. A physiological test of some sort is the only available means of measuring their effects. For this purpose it is necessary to find some animal which reacts in a constant manner to the poison, dying regularly within a certain time as the result of a given dose of toxin. In the case of the diphtherial toxins it is found that guineapigs are suitable test animals. It is possible to ascertain accurately the quantity of a particular specimen of poison which will invariably cause the death of a guineapig of a certain weight (250 grammes) on the fourth day. This is taken as the standard dose of poison, or “minimal lethal dose.” It is then necessary to find what amount of antitoxin is required to neutralize this dose exactly, and we find that equal quantities of a given antitoxin will perform this duty. A standard is thus set up. What is known as a “unit” of antitoxin is that quantity of antitoxic serum which will exactly neutralize 100 minimum lethal doses as above defined, i.e. an amount of which a one-hundredth part will prevent the appearance of any morbid symptoms in a guineapig, if injected along with the minimal dose of toxin which would otherwise kill it within four days.

In the case of an antibacterial serum the matter is rather more complicated. We may take as an example a serum which destroys cholera vibrios. Such serum, if injected into the peritoneal cavity of a normal animal along with a loopful of virulent cholera organisms, will rapidly

cause their disintegration; but, on the other hand, the bacteria, if injected into the abdomen of an animal (not immune) without any protective serum, rapidly multiply and kill the animal. In testing the strength of a serum different portions are diluted for the sake of accuracy, to (say) 1 : 100 and 1 : 1,000. Two guineapigs are taken, one of which receives 1 c.c. of the first dilution along with a loopful of a virulent culture of cholera vibrios, while the other receives the same quantity of the second dilution with a similar loopful of the bacteria. Within forty minutes search is made in the peritoneal cavities of the animals to see whether the vibrios therein are flourishing or are disintegrating. If the smaller dose of serum has failed to kill them, while the larger one has done so, further experiments are made to determine the exact quantity of serum which just suffices for the purpose; if the lower dose has proved sufficient, then still smaller quantities are tried, and that dilution of the serum which is finally determined (e.g. 1 : 500) is used to indicate the valency of the antiserum. Owing, however, to the difficulty of estimating the exact number and the virulence of the bacteria introduced, antibacterial serums cannot be standardized with the same precision as antitoxic serums, so their value is very rarely referred to in terms of "units."

#### ADMINISTRATION OF SERUM

**Early administration.**—The symptoms of an infective disease are due to the effects produced on the cells and tissues of the body by the toxins of its specific micro-organism, and consist in the resulting perversions of function; while the action of an *antitoxic serum* is to neutralize the poison circulating free in the blood and lymph, although it does not prevent the growth of the bacteria or exercise any restraining effect on them. Now, as the bacteria pour out a constant stream of toxin, and this is continually entering into combination with the side-chains (receptors) of the cells, it is most



important to introduce the antidote as soon as possible, before any great amount of mischief is done. If the disease has too long a start, the antitoxin may come too late to be of any service. The great principle, therefore, in giving antitoxin of any kind is to give it at the beginning of the disease, at the earliest possible moment. In the case of diphtheria, statistics show conclusively that the power of the remedy over the disease varies directly with the promptitude of its administration, while in the case of tetanus there seems reason to doubt whether it is not already too late, in man at all events, to use the antitoxin when the malady has declared itself. (*See pp. 114 and 146.*)

**Large dose.**—A second principle is to administer a large initial dose, since we do not in any case know the amount of toxin which has to be counteracted, and the supply of the latter is constantly increasing, whereas the remedy is given all at once in a single dose, and is not in any case repeated for some hours afterwards. There is also the possibility that the presence of a very large quantity of the antitoxin may tend to withdraw from the cells any poison which has already united with them. Similarly, we ought not to hesitate to repeat the dose, if it seem in the least necessary. It is better to err on the side of giving too much than too little. The danger of producing anti-antitoxin or anticomplement, previously alluded to (p. 37), does not seem to exist in practical therapeutics, though it might suggest the advisability of giving quite small doses of serum for prophylactic purposes. If, however, the previous administration of serum to a patient suggest the danger of anaphylaxis, the following method, devised by Besredka,<sup>1</sup> may be put into practice. Small quantities (1, 3, or 10 c.c.) of diluted serum (1 : 10) are injected intravenously at short intervals of a few minutes. After another similar short interval a moderate dose of the diluted serum (25 c.c.) is injected ; after which, allowing an interval of twenty minutes, a full dose of undiluted serum

<sup>1</sup> Internat. Congress of Med., 1913

can be administered by any route desired, without fear of anaphylactic symptoms ensuing.

**Fresh serum.**—Thirdly, it is important that the serum which is used should be as fresh as possible, as there is evidence that the remedy tends to deteriorate in course of time. How long the different serums may retain their specific powers is not yet definitely settled, and in the case of diphtherial antitoxin it seems probable that it may remain effective for at least two years. But there has been shown to be a slow process of deterioration at work in all cases, so that, in order to be on the safe side, it is well to use only quite fresh serum. If this is not to be obtained, or only after some delay, an older brand should be used rather than none at all, in preference to delaying unduly the administration of the initial dose. The same rule also applies to vaccines, which should be used fresh.

**The syringe.**—The syringe for hypodermic injection of serum should be of a capacity of not less than 10 c.c., as this is the dose of serum usually given, and it is inconvenient to refill the syringe during the administration of the dose. On the other hand, syringes of 20 c.c. capacity and upwards are heavy and clumsy to handle. It should preferably have a glass piston (Fig. 9), as this can be more readily sterilized than those provided with an ordinary leather washer. The latter may, however, be made of asbestos or of indiarubber. The needle should be longer than that of a common hypodermic syringe;  $2-2\frac{1}{4}$  in. is an adequate length. The bore of the needle need not be large, as the serum is perfectly fluid, and will pass readily through any hollow needle. It is unnecessary cruelty to employ the large-bored instruments often supplied, as they cause considerably more pain, and it is an advantage rather than otherwise to give the injection slowly. The serum at first causes a slight swelling at the point where it is injected, but this soon subsides. Its diffusion may be aided by a little massage of the part, but this is quite needless in the majority of instances. If a second injection is required, it may be



given at the point corresponding with the first, but on the opposite side of the body. If a series of doses is necessary, rows of punctures may be made in lines up the two sides of the abdomen.

In young infants the small size of all the parts must be borne in mind, so that the needle may not be inserted unduly far. Cases are recorded in which the pleura has been punctured in the process of injection in the flanks, and gangrene of the lung has ensued with fatal result.<sup>1</sup> With ordinary care no fear of injury to the youngest baby need be entertained. In the case of restless patients or young children it may be advisable to interpose a rubber coupling

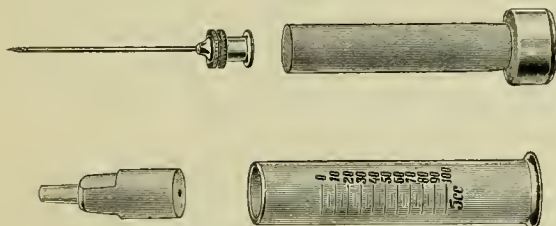


Fig. 9.—All-glass serum syringe; readily sterilized by boiling, after separation of the component parts.

between the needle and the barrel of the syringe in order to lessen the risk of breaking the former in the skin-puncture.

The syringe and needle must be carefully and thoroughly boiled<sup>2</sup> just before use in every case; and should be allowed to cool somewhat before the serum is drawn into them for use, to ensure that the temperature of the instrument is not such as to cause any coagulation of the albuminous fluid, whereby the needle might be blocked. After use the syringe and needle should be washed through, first with cold water and then with some volatile antiseptic, such as

<sup>1</sup> R. G. H. Tate, *Dublin Journ. of Med. Science*, April, 1900, p. 271.

<sup>2</sup> In the all-glass syringe the parts should be separated before boiling, to obviate danger of cracking.

ether or alcohol, and dried before being returned to the case. . . .

**Local use of serums.**—In some diseases which are characterized by distinct local lesions it has been advised to inject the serum in the neighbourhood of these, in order to procure a local effect. An antibacterial serum has been used in the form of lozenges in cases of diphtheria, in addition to the use of antitoxin subcutaneously. Tetanus antitoxin has been used to saturate the dressings of the infected wound, and the serum, dried, powdered, and mixed with chloretone or some inert powder, may be used to dust on the lacerated tissues at the seat of infection. In erysipelas good results have been obtained by injections of antistreptococcic serum into the periphery of the inflamed area; and in plague it has been recommended to make the injection into some part of the skin which is drained by the lymphatics leading to the bubo.

**Subcutaneous injection.**—Antitoxic serum is in general administered subcutaneously. It is immaterial what spot is selected for the injection; the sides of the abdomen are the favourite localities, as a rule, especially the skin near the groin. The back, between the shoulders, is equally convenient, but in the (unlikely) event of an abscess forming at the point of injection, as the result of some failure in antisepsis, the lesion would add more to the discomfort of the patient in this situation than if it were on the front of the body, where it would not interfere with the ordinary dorsal position of rest. The skin should be first washed with ether-soap and water, and then with some antiseptic, such as 1 : 20 carbolic-acid lotion, or better still with ordinary ether, which is then allowed to evaporate; or the skin may be painted with a solution of iodine in chloroform or alcohol (3 per cent.). The needle is passed quickly through the skin into the subcutaneous tissue, and the fluid is injected fairly slowly. The puncture is then sealed with a "scab" of collodion.

**Oral and rectal administration.**—Administration

of antitoxin by the mouth is of very doubtful efficacy (p. 32). If it is desired to give serum by this route, it may be mixed with 4 or 5 oz. of warm, not hot, soup or broth (100° F.), and thus effectually disguised. Rectal injection is advised by Marmorek in the case of his anti-tubercular serum, and has been employed by some physicians in the case of diphtherial antitoxin (*see* pp. 340 and 117). For this purpose the serum may be diluted with normal saline solution to a convenient bulk (say 2 oz.), and given by means of a bulb syringe, like a nutrient enema. It should be given at body temperature (98° F.) to facilitate its retention and absorption.

**Intravenous injection.**—In severe cases it has been recommended to give the remedy intravenously, in order that it may be more quickly absorbed and so manifest its effects more speedily.

For the purpose of intravenous injections, in the case of a child, some recommend a general anæsthetic, and for an adult cocaine-anæsthesia, since some have, on occasion, found it necessary to cut down upon and dissect out the vein. We, however, are of opinion that neither local nor general anæsthesia is necessary.

The vein usually chosen is either the median basilic or median cephalic at the bend of the elbow ; sometimes one is driven by circumstances to select a superficial vein of the forearm or hand, or else of the leg. The preparation of the patient is generally a simple matter, especially when one is able to utilize a vein at the bend of the elbow, for in this situation the skin is delicate, hairless, and easily cleansed. The patient should be lying in bed ; and the arm, bared to the shoulder, should rest on a clean towel on the bed by the patient's side, or, better still, be drawn out at right angles to the body. The skin must be thoroughly washed with soap and water ; scrubbed with ether ; more ether poured on and allowed to evaporate ; and finally the site for operation covered with a piece of lint saturated with ether. If iodine solution is used it should be washed

off as thoroughly as possible with ether, otherwise the position and course of the vein may be obscured by the brown discoloration of the overlying skin. If the vein chosen is covered by hairy skin, the part must be washed up with ether-soap and carefully shaved, the skin scrubbed up with lysol, and then, the lysol having been thoroughly washed off with large volumes of ether, finally covered with a pad saturated with ether.

The vein should be rendered prominent by compressing it above the bend of the elbow (this may be done by the thumb of an assistant), and the needle introduced through the skin into the subcutaneous tissue at a slight angle with the plane of the vessel. Then the direction of the point is slightly altered, and it is thrust through the wall of the distended vein. As soon as blood appears in the barrel of the syringe, the assistant releases the pressure on the vein; then the piston should be slowly and steadily pushed down, and the dose of serum injected into the vein. As the opening into the vein is small, pressure alone suffices to stop hæmorrhage.

The serum should be warmed to the temperature of the body before it is used intravenously; and if it exhibit any undue opacity or deposit, it should be strained through sterilized muslin before it is drawn into the syringe for injection.

In order to avoid any risk of injection of air into the circulation, it is necessary to see that the needle is full to the end with the serum before it is passed into the vein. There is in reality no danger to be apprehended if only a bubble or two of air enter a vein; and in vessels at a distance from the heart there is not a sufficient negative pressure to suck air in, apart from any injected. Still, care should be taken in this respect, and a finger may be placed over the vein on the central (cardiac) side of the point punctured, to prevent any possibility of mishap.

**Intracerebral injections.** — Some recommend the intracerebral route for the injection of antitetanus or anti-

meningococcic serum. In our opinion, and in view of the possible results of injury to the cerebral tissues, this method should be employed only as a last resource.

For the purpose of intracerebral injection, it is advisable to prepare a specially strong serum<sup>1</sup> by dissolving the solid (dried) substance in half the usual quantity of distilled water, or by evaporating the liquid serum to half its bulk at a low temperature *in vacuo*.

The procedure to be adopted is as follows: A line is drawn from one auditory meatus to the other, across the vertex of the skull. From the point at which this meets the midline a second line is drawn to the outer angle of the orbit. The middle point of this last line gives the site for the injection (*Roux's point*). An incision is made in the scalp, and a small trephine is employed to remove a piece of the skull. Abbe<sup>2</sup> says that the operation can be done satisfactorily under cocaine-anaesthesia. There does not seem, however, to be any advantage in this over chloroform, as the latter will control any spasm which may occur during the operation, while cocaine will not. An opening is made in the dura mater, and a blunt needle is thrust into the cerebral substance. The serum must be very slowly forced into the brain, 5 c.c. being enough to use on each side. It would seem better to endeavour to inject the antitoxin into the actual cerebral substance rather than into the lateral ventricle, as the latter method is practically equivalent to subdural injection, which can be more easily carried out, if it is desired, externally to the brain.

An actual trephine-opening in the skull is not necessary for the intracerebral injection, which can be given through a simple hole bored with a drill. The large cerebral sinuses must, of course, be avoided in these procedures, and a blunt needle is preferable, in order to avoid wounding the smaller vessels in the substance of the cerebrum.

A procedure similar to the above may be adopted

<sup>1</sup> Church, *New York Med. Journ*, Dec. 17, 1898.

<sup>2</sup> *Op. cit.*

for subdural injection, if it be decided to give this intracranially, but the method by spinal puncture is in all probability as effective.

**Intrathecal injections.**—The introduction of the antiserum into the spinal canal after lumbar puncture is essential in the treatment of tetanus and meningitis. In carrying out this method the patient should be arranged in bed in the semiprone position, on whichever side is more convenient to the operator, the head being slightly raised on a pillow. It is advisable to bend the knees and flex the thighs on the abdomen, so that the vertebral column is well arched, and to hold the patient firmly in this position. The site of operation for lumbar puncture is a 10-cm. (4-in.) circle of the skin of the back, having its centre over the spinous process of the fourth lumbar vertebra. This central point can be identified by the simple method of counting the vertebrae downwards, or by the help of a line joining the highest points of the iliac crests, which thus crosses the fourth lumbar vertebra. Adjust either the bed or the light so that the field of operation is well lighted; then prepare the skin, over the area already indicated, in the same way as at the bend of the elbow for the intravenous injection. The all-glass syringe, sterilized as previously described, must be provided with a somewhat longer needle (from 6–7 cm. long for children, to 9 cm. long for obese adults) than that used for intravenous injection. The needle is introduced vertically to the surface of the skin, into the interspace between the third and fourth lumbar vertebrae, and pushed on between the laminae into the spinal canal; and fluid is carefully withdrawn, so long as an abnormal pressure is noted, or, if no obvious pressure exists, until a rather larger volume has been abstracted than it is intended to replace by serum. The syringe is then detached from the needle, the latter being left *in situ*, a second syringe containing the antiserum, previously warmed to body temperature, being adjusted in the place of the first syringe, and the injection



made slowly into the spinal canal. In withdrawing the cerebro-spinal fluid, and also in injecting the serum, a careful watch should be maintained over both the pulse and respiration; indeed, Sophian<sup>1</sup> has suggested that in the course of intrathecal injections of serum, especially where it is desired to introduce a large volume of fluid, the blood-pressure should be recorded throughout the operation, and the injection stopped when the pressure falls to any appreciable extent (e.g. a drop of 20 to 30 mm. Hg). When the injection is completed, the needle should be withdrawn from the body, and the site of puncture sealed with a dressing of gauze and collodion or with a small piece of adhesive strapping.

**Intramuscular injection.**—It was shown by Meltzer and Auer<sup>2</sup> that solutions of salts were more rapidly absorbed into the system when injected into the muscles than when given hypodermically; and Morgenroth<sup>3</sup> found that the same was true of colloid solutions, such as serum. Practical use of this mode of administration was first made by Neisser and his assistants at Stettin, and since then many physicians have preferred this route. Rolleston and MacLeod<sup>4</sup> in this country speak highly of it, holding that this method should supersede all others, since not only is it simple to carry out, but it is less painful and less liable to cause abscesses than the subcutaneous method, while it is more rapid in its effects, and has the advantage over the intravenous route that the antitoxin is less rapidly excreted (or destroyed). The site of injection may be either the gluteal muscles or the vastus externus of the thigh.

#### OCCASIONAL ILL EFFECTS OF SERUM

**Effects on man of the serum of the lower animals.**—It is probable that the blood-serum is not

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1912, p. 843.

<sup>2</sup> *Journ. Exp. Med.*, 1905, vii. 59.

<sup>3</sup> *Berl. klin. Woch.*, 1907, xlv. 1349.

<sup>4</sup> *Brit. Journ. Child. Dis.*, 1914, xi. 298.

identical in composition in any two species of animals. We have already alluded (p. 11) to the poisonous effects produced in mammalian animals by injection of eel's serum, by which an actual hæmolysis is brought about. Other serums possess varying degrees of toxicity. Anti-toxic and immune serums are necessarily prepared from the blood of the lower animals, and the horse is usually chosen for the purpose on account of its size, which enables a considerable quantity of blood to be drawn at a time, as well as owing to the comparatively innocuous nature of the serum of this animal in its action on man. The injection of normal horse's serum into man may, however, be followed by certain results of an unpleasant, and at times even dangerous, character. It is found that the serum of some horses induces such results in larger measure than that of others. The fact that a horse has been immunized to a certain toxin or organism does not seem to have anything to do with the production of the symptoms referred to; the peculiarity resides in the serum of the animal, and is uninfluenced by the matters used for inoculation. It is also possible that an idiosyncrasy on the part of the patient injected may be the origin of some of the ill effects noticed. In such cases the hypothesis that the patient has become sensitized by the previous ingestion of horseflesh (which on the Continent of Europe is a fairly common article of diet) has been advanced. More frequently seen, and more severe, are the disturbances which may follow a second or subsequent injection of serum, owing to the supervention of a state of anaphylaxis or increased sensibility to the protein of horse-serum. To meet this danger both the ox and the ass have been utilized for the production of antidiphtherial serum to be used for patients possibly already sensitized to horse-serum; but it would appear simpler to adopt Besredka's method of anti-anaphylactic vaccination (p. 47) in such cases. Anti-anthrax serum is derived from the mule, and anti-pneumococcic serum from the goat.



**Nature of symptoms.**—As the most frequently used serum is the diphtherial antitoxin, it is chiefly in the case of this remedy that ill effects have been observed. They consist in cutaneous eruptions of various kinds (pp. 128-30), pains with some swelling and tenderness in the joints, and occasionally rise of temperature and feeling of illness. A good deal of itching is frequently met with at the site of injection. The rashes appear, for the most part, some time after the administration of the serum (second week), and are of the type known as erythema multiforme, i.e. they present many different appearances—erythematous, urticarial, scarlatiniform, morbilliform, etc.—but all are essentially conditions of hyperæmia and escape of serum into the tissue-spaces in varying proportions. Sometimes the hyperæmia predominates (erythema, etc.); sometimes the escape of serum (urticaria). In a very few instances more serious effects have ensued. Thus Rauschenbusch<sup>1</sup> records the case of a child who received a prophylactic injection of antitoxin, and who was seized with “giddiness, faintness, vomiting, and cutaneous irritation, with urticarial wheals, within a few minutes of the injection.” She remained ill for some hours, but was nearly well on the following day. Actual death may occur. A few instances have been recorded after the use of diphtherial antitoxin (p. 126), and the present writers have seen a case in which an injection of antistreptococcic serum in a patient suffering from pernicious anæmia was quickly followed by coma and death. When, however, we consider the enormous number of injections of serum of all kinds that are given, the number of fatal cases reported<sup>2</sup>—and it is probable that scarcely a single one of such fatalities escapes record, from its very rarity—becomes almost infinitesimal.

<sup>1</sup> Quoted by Durham, art. “Antitoxins,” in “Quain’s Dictionary of Medicine,” 1902.

<sup>2</sup> Some of these fatalities are probably attributable to the existence in the child of the “status lymphaticus,” in which sudden death may follow the most trivial injuries or operations.

The risk is much less than that of the smallest surgical operation, and can be entirely neglected in the presence of any real illness or even danger of infection.

**Mode of obviating ill effects.**—Ill effects appear to be associated to some extent with the amount of serum used for an injection, a large dose being more likely to be followed by rashes, etc., than a small one. There is thus reason to hope that the occurrence of these symptoms will become less and less frequent with the course of time, since, as it becomes possible to prepare serums of increasing anti-toxic strength, smaller doses will be required to produce the desired effects. Thus diphtherial antitoxin has been prepared containing as much as 1,500 units to the cubic centimetre, a comparatively small quantity of such a potent remedy being necessary for any one injection. Attempts have also been made to separate the antitoxin from the rest of the serum by precipitating the globulin and utilizing this for injection. Certain horses whose serum exhibits specially toxic qualities should not be used for the preparation of serum. Horse-serum is said to be most toxic when freshly drawn, and gradually to lose some part of its irritating qualities. Heating the serum to 60° C. also diminishes its toxicity, while previous administration of calcium chloride or lactate to the patient diminishes the risk of exudative phenomena (urticaria, etc.). Besredka<sup>1</sup> suggests the measurement of the toxicity of serums by estimating their power of inducing anaphylaxis. His method of avoiding the occurrence of anaphylaxis, which consists in the administration of a small quantity of serum and thirty minutes later injecting the full dose, has already been described (p. 47). It is advisable, if possible, to give a second dose of serum within a week of the administration of the first dose, thus anticipating the time at which anaphylaxis occurs, and possibly inducing anti-anaphylaxis. The latter, when once induced, appears to persist for an indefinite period.

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1907, xxi. 777.

## CHAPTER III

### BACTERIAL VACCINES: THEIR PREPARATION AND ADMINISTRATION

**Theory of vaccine<sup>1</sup> treatment.**—The administration of bacterial cultures for purposes of **prophylaxis** is a procedure which is easily explicable on ordinary principles, since the introduction of a dose of virus, which the individual is capable of overcoming by means of his natural powers of resistance, ensures a supply of antibodies being formed to resist subsequent attack by the infective organisms, or, more probably, educates the tissue-cells to throw off a large supply of such bodies on stimulation—since to confer active immunity it is necessary to introduce the specific virus into the individual to be immunized.

At the same time, it is evident that to introduce the infective material into a person or animal by the same channel as that by which infection is produced in the naturally acquired disease would merely induce the very condition from which it is sought to gain protection. Some other method must be selected. Several different ways of inoculation without conveying an actual attack of the disease are available:—

1. It is possible to inoculate attenuated virus, i.e. bacteria which have lost some of their power of producing disease. Attenuation<sup>2</sup> may be brought about (*a*) by

<sup>1</sup> The word vaccine is used on the analogy of the original discovery by Jenner, the principle being the same in the modern procedures. Wright has defined a vaccine as any substance which induces in the organism an elaboration of protective bodies.

<sup>2</sup> The employment of organisms attenuated by passage through animals has been called “Jennerization”; the use of chemical and other methods of weakening their virulence, “Pasteurization.”

growing the germs under conditions unfavourable to their development: anthrax bacilli grown at a temperature of 40° C. lose much of their virulence, and Pasteur made use of this method for preparing a vaccine for the protection of animals against this disease; (b) by addition, to cultures of the organisms, of chemical substances inimical to their growth: thus tetanus toxin may be attenuated by means of iodine trichloride for the purpose of the first inoculations made in a horse in the preparation of an antitoxin; (c) by heating the cultures of the organisms, as in the case of the vaccines for black-leg in cattle; (d) by drying, as in the case of the spinal cords of rabbits used in inoculation against hydrophobia (but *see* below, 5); (e) by passage of the infective material through an animal which has a greater power of resistance to it than man, as in the case of small-pox, which in the cow takes the form of the mild disorder, vaccinia; (f) by passage through an animal which, although it is as sensitive as man or even more so, yet alters in some way the properties of the virus, so that it is less adapted to cause human infection: thus it is said that the virus of hydrophobia, after passage through a series of rabbits, although its virulence for these animals is immensely increased, is yet rendered less potent for mankind.

2. The dead bodies of the bacteria may be injected instead of the living germs. This method is adopted in Wright's antityphoid vaccination and in the treatment of affections due to staphylococci, gonococci, *Bacillus coli*, and other organisms.

3. The bacteria may be inoculated in some special way, different from that by which they normally enter the body to cause infection. Thus cholera germs may be injected hypodermically, instead of reaching the alimentary tract by the mouth, as they do in cases of natural infection. The bacillus of black-leg (*Rauschbrand*) may be inoculated subcutaneously or intravenously for purposes of protection, its natural seat being the muscles. The tail is sometimes

chosen as a site of inoculation in animals, as being colder than the rest of the body and poorly supplied with blood.

4. An injection of antitoxic serum may at first be administered along with the virulent organisms, so as to neutralize part of their toxins until the animal has gained for itself the power of manufacturing antagonistic bodies. This method has been used by Sobernheim in inoculation against anthrax, and by others against cattle-plague and swine-erysipelas.

5. A similar result may be attained by the inoculation of a very small number of virulent germs, so that the patient can overcome them naturally, whereas grave infection would be induced by a larger number of organisms. This is the principle of Hogen's vaccination against rabies, in which a diluted virus is employed. It is not improbable that this is practically the basis of Pasteur's method in that disease, a certain number of the infective agents—those present in the external portion of the spinal cord—being killed by the desiccation, rather than all those present being reduced in virulence.

6. The bacteria may be "sensitized," before injection, by treatment with the copula or immune body of their specific bactericidal serum, the complement being previously destroyed by heat. Besredka<sup>1</sup> has made use of this method in vaccinating animals against the organisms of plague, cholera, and enteric fever; and Barié<sup>2</sup> has employed a similar method in the case of rabies. It is claimed that by this procedure the primary disagreeable effects of vaccination are avoided.

In many cases (not small-pox) there appears to be a risk in undertaking protective injections in the presence of an actual epidemic of the disease, since during the first few days after inoculation the cells of the body tend to form an excess of receptors (p. 24); thus, by offering more points of attachment, the cells are rendered more susceptible to the

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 918.

<sup>2</sup> *Compt. Rend. Soc. de Biologie*, Dec. 5, 1902.

toxins of the disease ; whilst the cells have not yet formed such an excess as to cause the discharge of free receptors into the serum.

In diseases which have a comparatively short incubative period it is necessary to administer the vaccine before infection has occurred. In small-pox, vaccination at the time of infection may probably exercise an effect in modifying the disease, since the incubative period of vaccinia (about four days) is shorter than that of small-pox (twelve days). In hydrophobia the latent period is so long that it has been found possible to produce the immunity after the patient has been infected with the disease, but before the symptoms have appeared. This is the principle of Pasteur's antirabic inoculation, of the protective value of which there can now be no reasonable doubt (*see* p. 171).

The use of vaccine as **treatment** for existing infection is not so readily intelligible. It might be argued that as living germs were already present in the body, giving rise to poisonous products, the injection of more of the same kind of germ, dead or alive, would merely add to the intoxication and so intensify the disease.

But in the case of local infections the bacteria establish themselves at a particular spot in a tissue which has little resistance, i.e. little power of producing antibodies, and the injection of a vaccine in some healthy distant situation may stimulate the formation of such bodies elsewhere ; they may then be brought to the infected focus by the blood, provided that there is a free supply of lymph transuding into the affected part. Such an influx of lymph is aided by local hyperæmia, the production of which is the object of such therapeutic measures as fomentations and Bier's passive congestion.

In the case of a septicæmia, a disease in which the infective organisms are carried all over the body by the blood-stream, this argument has at first sight but little theoretical weight, and we have to admit both that the basis of the treatment is largely hypothetical and that



the results obtainable by this mode of treatment are still a matter of controversy. Vaccine treatment of enteric fever, pneumonia, puerperal septicæmia, and malignant endocarditis, for example, has not yet been established on a secure basis. So far, it can only be suggested in its favour, by way of theoretical support, that while bacteria are not apparently destroyed in the circulating blood, they may be present in this fluid without settling in those particular tissues which have the power of forming antibodies; whereas dead bacteria introduced under the skin may be carried to such tissues and stimulate them to activity, being also, perhaps, more easily dealt with by these tissues than are the living germs, owing to lack of virulence (absence of aggressins or of the power of forming a protective capsule). An alternative explanation, suggested by the experiments of Faginoli,<sup>1</sup> is that the mechanism of vaccine-therapy belongs to the field of anti-anaphylactic phenomena.

#### PREPARATION OF BACTERIAL VACCINES

For all practical purposes these vaccines may be regarded as suspensions containing intracellular toxins in combination with bacterial protoplasm, and the results obtained by their employment depend on the activity of these toxins and the quantity present in the vaccine. Such vaccines may contain (*a*) bacteria in the "living" condition, either unaltered, attenuated, or "sensitized" by combination with homologous immune body; or (*b*) bacteria which have been destroyed, in some cases after preliminary sensitization, by chemical reagents, heat, or other physical agency. A vaccine may be "autogenous," that is, prepared from that particular strain of the bacterium already producing the infection of the patient; or "stock," that is, one prepared from another bacterium of the same species, but already stored in the laboratory, or recently isolated from another individual suffering from an apparently identical

<sup>1</sup> *Il Morgagni*, Oct. 31, 1915.



infection. However, a few general principles may at once be laid down with regard to their preparation. In the first place, there is a consensus of opinion that as the passage of an organism through the body of each individual host modifies to a greater or less extent its biological characters (resulting in the establishment of a number of "strains" of that organism), the best results will be obtained by utilizing an autogenous rather than a stock vaccine. The truth of this principle is borne out by the experience of numerous observers, although it is more obvious in some instances than in others. For example, in an infection with *B. coli* it is futile to treat the patient with a stock vaccine; but, on the other hand, a chronic gonorrhœal arthritis will often rapidly improve under treatment with a stock preparation. In some cases the preparation of an autogenous vaccine is a matter of great difficulty, if not of impossibility, as in the chronic arthritis already instanced, owing to the difficulty of isolating the responsible organism; but it should always be attempted, although a stock vaccine may be utilized during the interval.

Secondly, the organism must be as virulent as possible, and to retain this character the subcultivations used in the preparation of the vaccine must not be far removed from the body of the patient. In other words, the isolation of the bacterium from the morbid material must be effected as rapidly as possible, and in as few generations as is consistent with obtaining it in a state of purity. Hence an extensive knowledge of the food-requirements of the various pathogenic bacteria must be brought to bear upon the technique of the process of isolation.

Next, the particular subcultivation intended for the production of the vaccine must be cultivated under "optimum" conditions as to composition and reaction of the medium itself, temperature and atmospheric surroundings, age, and so forth; and the emulsion prepared from this subculture must be made with an indifferent fluid, and must be perfectly homogeneous and capable of sufficiently accurate

standardization to ensure subsequent facility of dosage. Now, in the case of the "killed" vaccine, the vitality of the bacteria contained in the emulsion must be totally destroyed by a lethal agent causing the least possible alteration in the molecular arrangement of the bacterial protoplasm—that is to say, the power of vegetative multiplication must be effectually removed from the bacteria, but complete coagulation of the protoplasm should be avoided. If heat is employed to attain this end, it is necessary to know accurately the thermal death-point, in watery emulsion, of the organism under treatment, and to conduct the operation of killing the bacteria at that temperature for as short a period as will attain the desired end. Other agents are sometimes employed, such as a weak solution of carbolic acid, or lysol, or sodium fluoride. Finally, after killing the bacteria, it is customary to adjust the strength of the vaccine to some convenient, although empirical, standard, and to add sufficient antiseptic to ensure the continued sterility of the emulsion.

The actual process of preparing a vaccine is briefly as follows, it being understood that all apparatus and reagents employed in the process have been sterilized.

The organism responsible for some given infection, having been isolated from the lesion existing in the patient and identified, is planted upon a suitable medium, either in tubes to form tube-cultures, or better in a Roux or similar bottle to form "mass"-cultures, and is incubated under optimum conditions for such period of time as experience shows is calculated to yield the maximum number of vigorous living bacteria.

At the end of the cultivation period the growth is examined visually to determine its freedom from gross contamination, and by means of stained preparations to determine its purity. The culture proving satisfactory, 5 c.c. of a 0.1 per cent. saline (NaCl) solution are pipetted into the tube or bottle, and the growth emulsified as evenly as possible with the help of a glass or platinum rod. The

turbid emulsion is transferred to a stout glass test-tube containing a number of glass beads: this is then placed in some form of mechanical shaker, and agitated thoroughly for about a quarter of an hour.

**Standardization of vaccines.**—The amount of bacterial protoplasm present in every cubic centimetre of the emulsion is next estimated by weighing, or by counting the actual number of bacteria in a hæmacytometer chamber, or by Wright's method. Unfortunately there is at present no special method which has obtained general acceptance; indeed some workers appear to standardize these vaccines merely by visual inspection of the density of the emulsion. Wright's method is perhaps the one even now in most common use; it consists in taking equal volumes of blood from a normal individual and of the bacterial emulsion, mixing thoroughly, spreading in a thin film on a glass slide, fixing and staining with Leishman's or Jenner's stain, and then with the help of the  $\frac{1}{12}$ -inch oil-immersion lens enumerating the numbers of red cells and of bacteria respectively in some twenty-five separate "fields" of the microscope. From the numbers thus recorded an average is struck and the ratio the red blood-disks bear to the bacteria is estimated. Now, assuming that normal blood contains 5,000 millions of red cells per cubic centimetre, a simple sum in proportion gives the number of bacteria present in each cubic centimetre of the bacterial emulsion.

Having determined the numerical strength of the vaccine, a sufficient quantity of 0.1 per cent. salt solution is added to the emulsion to reduce the weight of protoplasm per cubic centimetre to some convenient fraction of a milligramme, or to reduce the numbers in each cubic centimetre to 1,000 or 100 millions, or to whatever quantity is selected as the standard, and the mixture is well shaken. The tube is then suspended for a period of thirty minutes in a water-bath running at a temperature corresponding with the thermal death-point of the bacterium employed for the vaccine (e.g. 59° to 60° C. for *Staphylococcus aureus*). After removal

from the water-bath, loopfuls of the emulsion are sown upon suitable media, and incubated, in order to determine the sterility of the vaccine. Finally, a small quantity of some antiseptic, such as 0.5 per cent. carbolic, 0.25 per cent. lysol, or 0.25 per cent. trikresol, is added to the vaccine, which is then put up for use in separate doses in small glass bulbs, the necks of which are sealed in the blowpipe, or in bulk in rubber-capped bottles. In the latter case the requisite dose is removed from the bottle by means of the hypodermic syringe, the needle of which is made to pierce the rubber cap through a drop of pure lysol, the orifice being subsequently sealed with rubber solution.

**Sensitized vaccines.**—A modification of this method of preparation was introduced by Besredka in dealing with vaccines<sup>1</sup> for prophylactic use against cholera, plague, and enteric fever, in which, either before or after sterilization by heat, the bacterial emulsions were mixed with their respective antiserums. In such a mixture, after allowing it to stand for some twelve hours, it could be shown that the bacilli had entered into combination with the specific copula. The bodies of the bacteria were then washed free from serum in several changes of physiological salt solution, and standardized as already described. Besredka claims that with these sensitized vaccines active immunity is produced in the inoculated individual almost immediately, and that with very little local reaction and practically no constitutional disturbance.

As the result of further research work, Besredka and Metchnikoff, having failed to immunize the chimpanzee with heated vaccines, were successful in their attempts when they substituted sensitized living cultures of the typhoid bacillus—an observation which prompted Broughton-Alcock<sup>2</sup> to apply the same principle to man for prophylactic vaccination, and subsequently to employ sensi-

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 916.

<sup>2</sup> *Compt. Rend. Acad. Sciences*, May 17, 1912.

tized living vaccines of the pyogenic cocci in the treatment of staphylococcic, streptococcic, and gonococcic infections. This method has now been extended to include the treatment of a large number of different infections in the human subject, due to various bacteria.

The difficulty of obtaining specific serums for the purpose of sensitizing the bacterial cultures has been overcome by Broughton-Alcock<sup>1</sup> by injecting the patient first with an ordinary vaccine (sterilized by heat) of the infecting micro-organism and, when specific amboceptors can be demonstrated in that individual's serum, using this serum to sensitize the living vaccine—a procedure to which the term “double method” has been applied.

From time to time various observers have advocated and used living untreated bacilli as vaccines. Strong<sup>2</sup> in Manila employed a living plague-vaccine as a prophylactic measure, without untoward consequences; and Bourke, Evans and Rowland<sup>3</sup> have administered living typhoid cultures therapeutically, with apparently good results.

**Toxins as curative agents.**—The intracellular toxins of the tubercle bacillus have been used by Koch as a curative agent, under the name of “tuberculin.” It was found that the injection of this substance caused a distinct reaction at the seat of tubercular lesions such as lupus, and that the inflammation thus produced seemed to act beneficially on the course of the disease. In the case of the “new tuberculin” the toxic bodies of the bacteria are dissolved and injected, with the view of strengthening the patient's resistance to the disease (*see* p. 273).

A similar preparation from the glanders bacillus, termed “mallein,” has also been employed therapeutically in a case of glanders in man, but without beneficial result.

A trade preparation consisting of mixed sterile filtrates

<sup>1</sup> Immunity Section, Seventeenth Congress of Medicine, 1913.

<sup>2</sup> Quoted by Marx, “*Diagnostik, Serumtherapie und Prophylaxe*,” p. 81.

<sup>3</sup> *Brit. Med. Journ.*, 1915, ii. 587.

from broth-cultivations of a variety of micro-organisms, such as staphylococci, streptococci, pneumococci, *B. coli*, *B. typhosus*, etc., is sold under the name of "**phylacogen**." This is stated to be of value in the treatment of many bacterial infections, including rheumatism. Fortified by the addition of filtered culture-fluid in which some specific bacterium has been grown, it is advertised for use in infections due to that particular organism—i.e. gonorrhœa-phylacogen for gonorrhœa. Such a hotch-potch is quite unscientific, and, as the organisms employed are those which are ordinarily incapable of producing any appreciable amount of exotoxin in broth-cultivations, phylacogen resolves itself into a solution of altered protein more or less toxic in itself, and its injection into the human subject is a procedure which is not only useless but under certain circumstances may be dangerous.

**Phenomena accompanying active immunization by vaccines.**—The immediate result of the introduction of a suitable dose of vaccine into the tissues of a patient is a fall in the amount of opsonin present in the serum, owing presumably to the linking-up of some of the available opsonin to the bodies of the bacteria introduced. This is termed the "negative phase" (Fig. 10), and occupies a period lasting from a few hours to a week or ten days, or in exceptional cases a fortnight or more. Its duration is increased by a larger dose, and reduced, or even eliminated altogether, by a smaller dose.

As a result of the stimulus provided by the successful use of a vaccine, fresh supplies of opsonin are elaborated and discharged into the serum, and the negative phase is succeeded by a "positive phase," during which the opsonic index rises slowly or rapidly to a maximum, after which it frequently oscillates slightly for a day or two, and then comes to rest, and a condition of "equilibrium" is established, in which the index is maintained at a higher level than it occupied before the injection, although even now not necessarily at or above the normal. This state of



equilibrium, after a period varying with different individuals, with the size of the dose, etc., declines either gradually or rapidly until it has fallen to, or below, its original position. A repetition of the dose of vaccine now causes a repetition in their entirety of the phenomena already detailed. If, how-

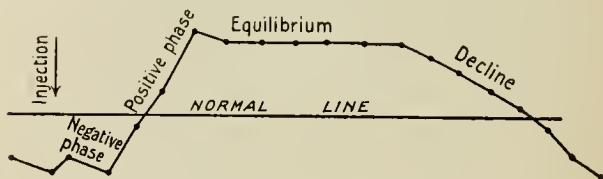


Fig. 10.—The opsonic cycle. (In this and the following figure the arrow indicates an injection.)

ever, a second dose of vaccine is administered during the negative phase induced by the first injection, a cumulative action is noted, and a second negative phase is superposed on the first: the opsonic index will then rapidly fall, perhaps with serious results to the patient (Fig. 11). On the other hand, a second dose injected at the highest point of the positive phase will not in most instances give rise to cumulation of positive phases (although in some infections

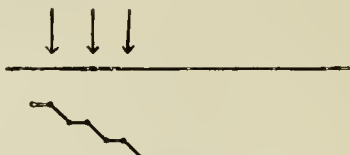


Fig. 11.—Opsonic index, showing rapid fall.

—for example, those due to the gonococcus, *Bacillus coli* etc.—this highly desirable end can be obtained). Usually such a procedure merely results in a shorter or less marked negative phase (Fig. 12). Practically it is found that good clinical results are obtained if the index oscillates about the normal level, provided that the greater part of the curve representing the movements of the index



is above normal (Fig. 13). Consequently, to obtain the best results by the aid of vaccines, subsequent doses should be injected towards the end of the period of equilibrium.



Fig. 12.—Opsonic index, showing less marked negative phase.

Endeavour should be made so to adjust the dose as to obtain the shortest negative phase compatible with the production of a positive phase lasting from five to seven days.

In many acute infections, however, it is of greater importance to reduce or eliminate the negative phase which

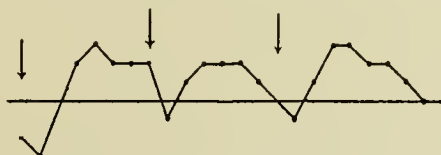


Fig. 13.—Opsonic index, showing oscillation about normal level.

follows the injections than to lengthen the positive phase ; and a dose must be administered so minute that the positive phase appears almost immediately ; and must be repeated before the transient positive phase has declined—sometimes within twelve hours (Fig. 14).

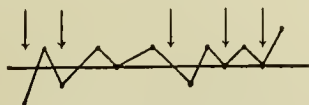


Fig. 14.—Opsonic index, showing immediate appearance of positive phase.

Similar observations have been recorded in connection with other antibodies, particularly agglutinins.

## ADMINISTRATION OF VACCINES

**General considerations.**—Practically every case of bacterial infection presents points of peculiarity, and although it is impossible to lay down any hard-and-fast rule for the dosage or administration of vaccines, a few general considerations can be stated as the result of experience accumulated by many workers during the past ten years.

The first essential is that the diagnosis should be accurate, and that full and complete information should be available as to the exact organism responsible for the infection, since the futility of treating a streptococcus infection with a staphylococcus vaccine is sufficiently obvious.

The estimation of the opsonic index is not absolutely essential for the successful conduct of treatment, careful attention to clinical signs and symptoms, temperature reactions, and local condition being in most instances sufficient guide for the administration of vaccine in the acuter forms of bacterial infection. Thus, during the negative phase, although no appreciable rise of temperature occurs (unless an excessive dose has been administered), the patient sometimes complains of not feeling well, and any local lesion that may be present is objectively worse—the discharge from a sinus increases in amount; in a cystitis there is an increase in frequency of micturition, and more pus is present in the urine; in furunculosis, a fresh crop of boils may appear, and so on—whilst during the ensuing positive phase a subjective sense of well-being is experienced, pyrexia diminishes, often rapidly, and clinically the improvement is marked. In the subacute and chronic types of infection the weighing machine is a useful adjunct to clinical observation.

When undertaking vaccine treatment without the assistance of the opsonic index, the initial doses must be small, in order to avoid the risk of a negative phase

excessive in amount or duration. Then, too, the age, weight, and general condition of the patient must to a certain extent be taken into consideration. Speaking generally, an emaciated infant or an enfeebled and elderly patient would receive a very much smaller dose than a well-developed and apparently vigorous adult. With acute and generalized infections the initial dose should be very small indeed, perhaps not more than one million bacteria, but in subacute infections it may well be ten times as large, and in chronic conditions a hundred times as large. Again, so long as improvement is maintained with any given dose, it may be stated in general terms that there is no object in increasing that dose. With most vaccines, however, it will be found that after a time any given dose is incapable of provoking a response equal to that at first obtained, and then it is necessary to alter the dose from that originally determined; and similar changes will be needed throughout the whole course of treatment.

It should be remembered, too, that a dose sufficiently large to provoke constitutional disturbance and pyrexia usually goes hand in hand with a negative phase of considerable extent. Pyrexia should, therefore, be an indication for making the ensuing dose very much smaller in size.

Carefully conducted surgical treatment should accompany the vaccine treatment throughout. Thus, whenever possible, an infected area should be immobilized, an infective focus removed, and so on.

**The syringe.**—The vaccine syringe should be of the all-glass type similar to that used for serum-injection, but of 1 c.c. capacity, graduated on the barrel in tenths and twentieths. The piston, if made of blue or amber glass, renders the process of subdividing doses an easy matter. The ordinary steel hypodermic needle is to be preferred to the platino-iridium needle, since as a general rule it is finer and takes a sharper point, whilst, being less costly, it can

be replaced frequently at no great expense. Syringe and needle are best sterilized by boiling immediately before use, though some prefer to draw up into the syringe boiling olive oil and then eject it.

**Site of inoculation.**—Theoretically, the dose of vaccine may be injected into loose subcutaneous tissue wherever situated in the body, but practice has shown that some sites—usually those poorly supplied with cutaneous nerves—are preferable to others; thus injections into the arm or leg, where the subcutaneous tissue is scanty and the movement of the underlying muscles frequent and unavoidable, tempting though such situation may be on account of the accessibility, should be sedulously avoided. Vaccines should not be given by the mouth or by the rectum. The best situations are the subcutaneous tissue underlying the anterior abdominal wall, or in the flank; at a point about an inch below the centre of the clavicle; below the point of the scapula; on either side of the middle line between the scapulæ; along a line about an inch above and parallel to the spine of the scapula; or at the base of the mammæ. The skin over the selected area should be washed with ether or acetone, or painted with tincture of iodine. Concentrated disinfectants such as pure lysol should not be employed.

The selected dose having been drawn up into the syringe, a fold of prepared skin should be pinched up between finger and thumb, the needle plunged boldly through it into the subcutaneous tissues and the vaccine ejected from the syringe, the needle quickly withdrawn, and the puncture again wiped with the antiseptic previously used. No dressing of the puncture is needed.

#### ADDENDUM

**Use of lactic-acid bacilli.**—Metchnikoff's suggestion, that by the ingestion of cultures of *B. bulgaricus* the more noxious denizens of the intestinal tract might be crowded out, led to a large number of experiments in the use of this treatment in a variety of alimentary

and intestinal diseases, but the results have not come up to the hopes that were first aroused. In obstinate conditions of disturbance of the intestinal functions, however, when other means fail, the method is worthy of trial.

Local use of cultures of lactic-acid bacilli has been recommended by North<sup>1</sup> in conditions of the mucous membranes. He reports very good results in cases of atrophic rhinitis (56 cases, 50 improved); his statistics of results in other conditions, such as otitis media, leucorrhœa and gonorrhœa, are not very striking. Two cases of acute diffuse peritonitis are stated to have been successfully treated by this means, which has also been employed for suppurating wounds and bruises. Persson<sup>2</sup> records successful results in 28 out of 36 gonorrhœal cases; and Newman<sup>3</sup> records the use of intravesical injections in the treatment of cystitis.

Lactic-acid bacilli have also been used as a spray with a view to dislodging persistent diphtheria bacilli from the fauces.<sup>4</sup> For this purpose a spray of staphylococci has also been employed, but this organism is pathogenic and may give rise to tonsillitis,<sup>5</sup> hence its employment would seem to be dangerous and unjustifiable.

<sup>1</sup> *Med. Record*, 1909, lxxv. 509.

<sup>2</sup> *Ibid.*, 1910, lxxviii. 534.

<sup>3</sup> *Lancet*, 1915, ii. 330.

<sup>4</sup> *Journ. Amer. Med. Assoc.*, 1913, p. 392.

<sup>5</sup> Davis, *ibid.*, 1914, lxi. 393.

## CHAPTER IV

### SERUMS AND TOXINS IN DIAGNOSIS

#### AGGLUTINATION TESTS

THE first observations on the agglutinating power which the serum derived from patients suffering from certain infective diseases exerts on the bacteria causing the condition were made by Gruber and Durham with regard to the reaction as it affects the bacilli of enteric fever. It was afterwards found that very many kinds of micro-organism were similarly affected by the serum of animals immunized against them, but it will be more convenient to discuss the rationale of the test in connection with the *B. typhosus*.

**Enteric fever.**—The reaction was first suggested as a practical test for diagnosis of enteric fever by Widal in 1896, although experiments in this direction had previously been made by Grünbaum: the latter were not published till after Widal's communication. The "test" may therefore fairly be called "Widal's," although he was not the discoverer of the phenomenon. A very large amount of experience is now available as to the occurrence of the "reaction" in cases of enteric fever.

At first it was thought that the mere fact that the serum of a patient possessed the clumping property was conclusive evidence that the disease from which he was suffering was enteric fever; but it was soon found that the serum of many normal persons was capable of producing the same effect. That derived from typhoid patients, however, is much more strongly agglutinative than normal serum, and will produce the reaction even if considerably diluted (e.g. 1:200). The test as at first described was

therefore modified, a serum diluted by mixture with nine times its volume of normal saline solution being employed.

It is now recognized that a dilution of 1:10 is not sufficient to exclude a number of cases in which the individual normally possesses a somewhat high agglutinative power without any present infection with enteric fever. A dilution of 1:50 is therefore taken as the lowest dilution from which to judge of the reaction of a serum in suspected enteric fever; if such a diluted serum causes agglutination within half an hour, the reaction is called positive.

Libman<sup>1</sup> states that a positive reaction may sometimes occur in high dilutions (1:500) when it is not present in more concentrated mixtures (1:20); he therefore recommends the use of two dilutions for each test (*see* p. 81).

**Mode of performing the Widal test.**—The blood of the patient may be obtained from either the finger-tip or the lobe of the ear. The latter is, perhaps, the better of the two, as it is less sensitive, and the blood flows quite as freely, if not more so. The skin should be cleaned up first with lysol or similar antiseptic, and afterwards with sterilized water: this precaution is not, however, absolutely necessary. The lobule of the ear is then firmly grasped with the fingers of the left hand, and a deep puncture is quickly made with a sharp surgical needle, or with a special instrument made for the purpose. A common needle will serve, if no other is available. The blood is collected, as it exudes, in a glass bulb drawn out at either end into a fine point; the ends being sealed in a flame after the blood is collected. In the tube coagulation takes place, and the serum which exudes from the clot is ready for use. It is advisable to dilute the serum itself before mixing it with the culture of bacilli, and not merely to use the latter for purposes of dilution; since the pure serum may produce some clumping on first coming into contact with the bacilli, before the whole is properly mixed, and errors may thus arise. For dilution of the serum,

<sup>1</sup> *Med. News*, Jan. 30, 1904, p. 204.



sterile salt-solution (0·6 per cent.) must be employed, since in the absence of salt the reaction may fail.

Several different ways of effecting the necessary dilution of the serum are employed. It is best to use, at all events for the higher dilutions, a graduated pipette, which saves time and trouble. Some sterile neutral fluid such as normal saline solution is used for the purpose. The broth-culture of the *Bacillus typhosus* must be a recent (eighteen to twenty-four hours old) and vigorous one, in which the bacilli are moving freely about. In older cultures an agglutinating substance is formed by the bacilli and diffuses out into the liquid; in such specimens the bacilli are found to have become clumped without the addition of any extraneous material, and are therefore unfit for use. The addition of a few drops of an old culture to a young and vigorously moving emulsion will produce agglutination. It is well to observe the condition of the culture before using it, in order to see what (if any) degree of clumping is already present.

When the dilution has been made and the bacilli added to it, a drop of the mixed fluid is placed on a cover-glass, and a hanging-drop preparation is made, and observed under the microscope with a medium-power objective. The cover-glass should be ringed round with vaseline or some similar substance, to prevent evaporation. A high power is not necessary; indeed, it may even be a source of fallacy to beginners, by leading them to mistake the small clumps which are present in almost all cultures for the larger masses which form as the result of the true agglutination. At first the bacilli can be seen moving actively about in all directions, but their movements gradually become more sluggish and finally cease, while the organisms may be seen to become aggregated into lumps or masses. If a true agglutination of the bacilli takes place, it will be seen that almost all of them have run together into masses, while any that remain free have lost their motility and remain stationary in the field of the microscope.

A time-limit is necessary for this test, and half

an hour is that usually taken. If within this time the bacilli have all, or nearly all, ceased to move and become massed together, then the test is said to be positive. Although the test is generally conducted at room-temperature (about 20° C.), the optimum temperature for agglutination is said by Weil<sup>1</sup> to be 60°–65° C.

The test may also be done macroscopically, by mixing the serum and culture in a watch-glass. A visible precipitate falls if the reaction is positive. According to Berliner and Cohn,<sup>2</sup> a star-like figure is seen in a watch-glass in half an hour at room-temperature.

Another way of making use of the agglutination reaction for diagnosis is to add a measured volume of serum to a known quantity of culture in a test-tube. If the former possesses agglutinative properties a precipitate forms in the tube, visible to the naked eye owing to the subsidence of the clumped bacteria to the bottom of the glass. This is known as the "sedimentation test" or the "precipitation test." It is also possible to cultivate organisms in the serum and to compare the appearances which they present with those of cultures in ordinary serum. In some cases the growth in the specific serum is characterized by clumping or by formation of chains or threads (Pfaundler's reaction).

McWeeney<sup>3</sup> has devised a special method of performing the test, by growing the bacilli in hanging drops, one with the serum to be tested, the other with normal serum. If the reaction is positive, the bacilli in this drop will be seen to form chains and to be non-motile, whereas in the "control" experiment they are separate and freely motile. The serum is added in the proportion of 1 per cent., and the slides are kept at 37° C.

Hewitt and Rowland<sup>4</sup> are the authors of a means of performing an exactly graduated quantitative test. The

<sup>1</sup> *Prager med. Woch.*, 1904, No. 19, p. 233.

<sup>2</sup> *Münch. med. Woch.*, Sept. 11, 1900.

<sup>3</sup> *Dublin Journ. of Med. Science*, Sept., 1898.

<sup>4</sup> *Brit. Med. Journ.*, 1900, i. 1015.

serum is received into capillary tubes, of which the thickness of the walls and the diameter of the lumen are measured under the microscope, while the length of tube which is filled by the serum is easily ascertained. In this way the exact volume of serum is calculated, and subsequent dilution is effected by measured proportional amounts of broth.

Ficker<sup>1</sup> has devised a method of performing the test with dead bacilli, specially prepared and suspended in an indifferent fluid, the nature of which has not been published. The serum to be examined is diluted (1 : 10) with saline solution, and mixed with the slightly turbid test-fluid. If the reaction is positive, the mixture becomes clear, a slight precipitate falling to the bottom. Ten to fourteen hours are allowed for the reaction to take place.

The value of this ("*Ficker's diagnostic*") as a test is confirmed by Meyer,<sup>2</sup> Ehrlam,<sup>3</sup> Sadler,<sup>4</sup> and others. It is, however, not so delicate as Widal's test performed in the ordinary way.<sup>5</sup> If further experience prove favourable, the discovery should afford a useful means of applying the test, as the dangers inseparable from living organisms and the trouble of preparing fresh cultures will be avoided. The preparation is said to keep well for at least nine months.

**Value of Widal's reaction.**—It was at first hoped that in Widal's reaction we possessed a certain test for the existence of enteric fever, but we now know that this is not the case. On the one hand, a certain number of undoubted cases of enteric fever fail to give the reaction at all. Fatal cases are from time to time encountered which never show any power of agglutination, but which

<sup>1</sup> *Berlin. klin. Woch.*, 1903, p. 1021.

<sup>2</sup> *Ibid.*, 1904, p. 166.

<sup>3</sup> *Münch. med. Woch.*, 1904, p. 662.

<sup>4</sup> *Berlin. klin. Woch.*, 1905, No. 10.

<sup>5</sup> Güttler, *ibid.*, 1904, Nos. 51, 52; Selter, *Münch. med. Woch.*, 1905, No. 3.

present post mortem the characteristic lesions of the disease. On the other hand, cases which are not enteric—e.g. paratyphoid fever—may exhibit a comparatively high agglutinative power (*see also* p. 231).

**Other diseases.**—The serum of patients suffering from other diseases may possess towards the corresponding bacteria as high an agglutinative power as that found in enteric fever, or even higher degrees. Thus, in **Mediterranean fever** it is quite usual for the serum of patients to clump the micrococcus in dilutions of 1 : 250, or even 1 : 1,000, though here, too, a dilution of 1 : 50 is recommended as a good practical working strength for diagnosis. The serum of **dysentery** patients may clump Shiga's bacilli in a dilution of even 1 : 1,000 in some instances. Posselt and Sagasser<sup>1</sup> consider that a dilution of 1 : 50, recommended by Shiga, is here not sufficient to secure an accurate diagnosis. The serum of a guineapig artificially immunized against colon bacilli may react with these organisms in a dilution of 1 : 25,000, while that of a typhoid-immunized horse may possess nearly equal strength.

**Group agglutination.**—The observers just quoted show that while a serum may normally possess a power of agglutinating several kinds of bacteria, the process of immunizing the animal against one kind of organism will raise the agglutinative power against the others, though not in equal degree. Thus the serum of a patient suffering from dysentery may possess an agglutinative power for *B. dysenteriae* of 1 : 300, while it may react with *B. typhosus* at 1 : 75, with *B. coli* at 1 : 30, and *V. cholerae* at 1 : 35. If examination were only made for its reaction with typhoid bacilli, an error of diagnosis might easily result. They therefore hold that it is necessary, before accepting a reaction as positive, to test the agglutinating power against several organisms. It need hardly be pointed out that, if such be the case, it adds considerably

<sup>1</sup> *Op. cit.* (*see* p. 15).

to the difficulty of making the test, and thereby detracts greatly from its value for everyday use.

**Persistence of reaction.**—The agglutinative power remains present in the serum long after the infection which led to its appearance has subsided. Hence not only do convalescents from, for example, enteric fever react to Widal's test, but also persons who have suffered from the disease in previous years. How long the property remains is not known for certain; probably it varies in different individuals, and perhaps according to the severity of the attack. In the case of enteric fever it has been proved to persist for over eight years (French and Louisson), and after dysentery it has been found to last for at least a year in some cases (Kruse). It is suggested that the duration of agglutinative power corresponds with that of immunity to the disease, but this cannot be considered proved as yet.

A drawback to the use of the test as a means of diagnosis lies in the fact that it does not appear quite at the beginning of the illness, at which time it is most needed as an aid to diagnosis. Thus, in enteric fever it cannot be relied upon to appear before the second week of the disease; in plague it may be absent until convalescence. In a person who has been *inoculated* against typhoid fever the serum becomes agglutinative, and Widal's reaction as applied to a chance sample is therefore misleading if employed for the diagnosis of obscure febrile conditions. In such cases a series of observations must be carried out, and the extreme limit of agglutination determined on each of several successive days. In actual typhoid infection the limit will vary, but if the reaction be due to prophylactic inoculation the agglutination will remain to all intents and purposes steady. In dysentery the reaction is often wanting in mild cases, according to Shiga. This author holds that the agglutinative power in any case bears a direct proportion to the severity of the infection—a contention that will not hold good in all cases, for a very mild case of

Mediterranean fever seen by the authors exhibited a complete reaction when tested against *M. melitensis* in a dilution of 1 : 500,000, and fatal cases of enteric frequently fail to elaborate any demonstrable quantity of agglutinin.

#### DIAGNOSIS BY OPSONIC ESTIMATION

The estimation of the opsonic index may be of considerable assistance in the diagnosis of obscure conditions, for an index well above 1·2 to any given organism points in no uncertain manner to infection by that particular bacterium. Moreover, when, for example, tuberculosis is suspected and the index at the first estimation is within the normal range (e.g. 1·2 and 0·8), a series of observations at frequent intervals will usually reveal the characteristic movements (*see* p. 70) of the patient's tuberculo-opsonic index, if the condition is actually due to the *B. tuberculosis*; while, if it is due to some other cause, the index will remain practically steady, or, at any rate, its curve will not show marked excursions from the normal line. Occasionally, however, it may be necessary to extend the observations of the opsonic index over a considerable period of time before the resulting curve exhibits characteristic features.

In order to obviate the delay involved by watching the natural movements of the opsonin curve, the metabolic products of the tubercle bacillus, in the form either of an auto-inoculation or of tuberculin (T.R.), may be utilized. Thus, supposing that the case is one of suspected tuberculous disease of the knee-joint, or of the kidney, exercise of the affected joint, either by passive manipulation or by its use in active movement in the one case, or palpation and massage of the affected kidney in the other, will lead to the discharge of tubercle bacilli or of their products into the circulation, and will produce all the phenomena described as following the subcutaneous injection of a suitable vaccine—in this case tuberculin—i.e. negative phase, positive phase, and so on, and will so confirm the diagnosis. If, on the other



hand, the affection is not of a tuberculous nature, no appreciable movement of the tuberculo-opsonic index will take place.

If, however, it is impossible or undesirable actively to interfere with the actual focus of infection, a diagnostic dose of tuberculin (e.g. 0.0002 mg. tuberculin, T.R.) may be injected subcutaneously, and its effect upon the amount of opsonin present in the blood-stream noted. For this purpose samples of blood should be taken immediately before, and also one, four, twelve, twenty-four, and forty-eight hours after, the injection. All these specimens may be examined at one and the same time (using the same batch of tubercle emulsion and of "washed" cells), and all compared with the same control "normal" serum, when estimating the opsonic content of each blood-sample. With a small dose such as this, in the case of the tuberculous lesion, the immediate transitory rise or spurious positive phase, the negative phase, and the true positive phase may be confidently anticipated in rapid sequence.

**Method of determining the opsonin-content of the blood-serum.**—For this estimation it is necessary first to prepare a quantity of living human leucocytes from some indifferent source (either the patient or some normal individual), from which the plasma has been completely removed by receiving the blood, as it exudes from a needle-puncture, into a weak (1.5 per cent.) solution of sodium citrate in normal saline, centrifugalizing the mixture thoroughly, pipetting off the citrated plasma, adding normal saline solution to the deposited mass of red cells and leucocytes, mixing thoroughly, and again centrifugalizing. A repetition of this "washing" with normal saline and the subsequent removal of the supernatant fluid leaves a sufficient quantity of leucocytes and cells entirely freed from the fluid (which possibly contained excess of opsonin) in which they were originally suspended.

Next, a small quantity of the blood to be tested is collected in a small pipette and allowed to clot, and the



serum is separated. Similarly, the serum from the normal individual or "control" is prepared. Finally, a homogeneous suspension of the test-bacterium is prepared by emulsifying some of the bacterial growth from a young agar-culture in distilled water, and carefully centrifugalizing in order to throw down any possible clumps or masses of micro-organisms. By means of a suitable measuring pipette—for example, a Pasteur pipette furnished with an india-rubber teat, as introduced by Wright (Fig. 15)—equal quantities of washed cells, of bacterial emulsion, and of the patient's serum are taken up and thoroughly mixed, and the mixture incubated at body-temperature for fifteen minutes. At the end of this time, after preliminary manipulations to ensure that there is thorough incorporation of the mixture,

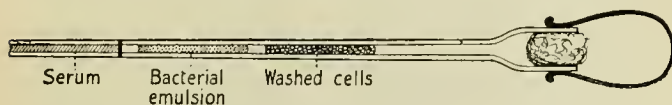


Fig. 15.—Measuring pipette for opsonin-estimation.

blood-films are spread therefrom on an ordinary glass slide, fixed, and stained. A second mixture, in which the normal serum is substituted for the patient's serum, is prepared and incubated in an identical manner. The two slides are examined microscopically, and the number of bacteria taken up and ingested by the first 50 consecutive polymorphonuclear leucocytes encountered in each film is noted (Fig. 16). The ratio between the resulting sums is then expressed in the form of a fraction, of which the total content of the 50 cells mixed with the patient's serum forms the numerator, and that of those with the normal serum the denominator. This fraction is, however, usually expressed as a percentage of unity—which is represented by the normal serum. Thus, supposing 150 bacteria were counted in 50 cells of the preparation made from the patient's serum, and 100 in a similar number in the preparation from the normal control, the index would

be represented by  $\frac{1.5}{1.0}$  or 1.5. If, however, the specimens chosen had given the figures 60 and 90 respectively, the fraction would have been  $\frac{60}{90} = 0.66$ .

**Value of opsonic determinations.**—The accuracy of opsonic determinations practised according to Wright's method depends on the postulates that all polymorphonuclear leucocytes, whatever their source, are equally active in ingesting bacteria, and that the opsonic power of the serum is constant in normal healthy persons. The former supposition is contrary to what obtains in the case of

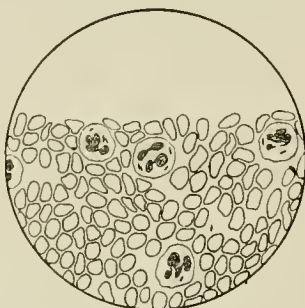


Fig. 16.—Edge of blood-film preparation, showing phagocytosis of bacilli by polymorphonuclear leucocytes in the presence of serum containing opsonin.

other vital phenomena, in all of which differences are found to exist between individuals; and the uniformity of leucocytic action has been questioned on experimental grounds by several observers (Ledingham,<sup>1</sup> Rosenau,<sup>2</sup> Potter<sup>3</sup>). Many series of experiments have been performed to elucidate the constancy of the opsonic content of normal persons. Bulloch<sup>4</sup> found that the tubercular

<sup>1</sup> *Journ. Infect. Dis.*, iii. 683.

<sup>2</sup> Quoted by Dean, *Brit. Med. Journ.*, 1907, ii. 1409 (with full literature).

<sup>3</sup> *Journ. Amer. Med. Assoc.*, 1907, xlix., No. 22.

<sup>4</sup> *Lancet*, 1905, i. 160.

opsonic index in healthy persons might vary between the extremes of 1·2 and 0·8, the majority of individuals being practically equal (1·0). Such a difference seems small in itself, but it is clear that a considerable percentage of error may be introduced thereby into individual calculations. Thus, if a normal serum with an index of 1·2 be taken as the standard, the normal serum at the other extreme of the scale will be found to have an index of 0·66 ; while conversely, if the lower normal index be taken for comparison, the higher will show an index of 1·5. Both 0·66 and 1·5 would be held to be pathological and to point, the former to the possibility, and the latter to a probability amounting for practical purposes to a certainty, of tubercular infection.

This difficulty is, however, overcome in practice in one of two ways, either by using as the normal serum the "pooled" serum of several normal individuals, or by always taking the serum from the same healthy individual (standardized from time to time against "pooled" serum) as the normal ; for the index of the normal serum, whether at one limit or the other of the normal range, remains practically stationary from day to day.

Doubt has been thrown on the accuracy of the ordinary method of estimating phagocytic activity by Fitzgerald, Whiteman and Strangeways,<sup>1</sup> who have shown that different workers may with the same serum obtain widely divergent results. For example, the following pairs of figures give extreme instances of such divergence, each pair representing the indices obtained from one specimen by different observers: 1·70-0·36, 1·92-0·68, 1·18-0·31. Further, if, on the same slide, successive series of 50 leucocytes are counted, very different numbers of enclosed bacilli are found. Thus, on the same slide in one instance, one series of 50 cells contained 119 bacilli, and a second series of 50 contained 74 only. In another instance the numbers were 150 and 71. Even when 100 cells were

<sup>1</sup> *Bull. of the Committee for the Study of Special Diseases*. Cambridge, 1907, i., No. 8.

counted instead of 50, the percentage difference amounted in one instance to 115. Hence these writers conclude that for accuracy of observation not less than 1,000 cells must be counted. Since this would involve an expenditure of time amounting to several hours for each slide, it is clear that, if these writers are correct, accurate estimations are beyond the sphere of practical use. Even this alternative is rejected by Greenwood,<sup>1</sup> who considers that counting 1,000 cells will not necessarily give greater accuracy than counting 25.

Even if it be admitted that accurate estimations of the phagocytic activity of a serum can be made, it remains to consider what the value of such an estimation is as a measure of a patient's degree of immunity. It is admitted, even by Wright himself, that the opsonins are not the sole means of resistance to bacteria; there are besides not only the agglutinins, bactericidins, and antitoxins, but also the resistance of the cells of the various tissues, which probably plays a very important part. Measurement of the opsonic power of the serum therefore throws light on only one factor in immunity. In earlier days the agglutinating power of the serum was used as an index of immunity, until time showed that it was untrustworthy. Similarly it cannot be accepted without more rigorous proof that the amount of opsonins present is a fair measure of the total resistance. In many instances the variation of the opsonic index does not correspond with the clinical course of a case, while it is known to alter in response to slight influences which cannot be supposed to affect the general degree of resistance. Thus, Latham<sup>2</sup> found that while the opsonic index in tuberculous individuals varied throughout the day inversely with the patient's temperature, a slight degree of bodily exercise in such a patient may notably reduce the index.

In spite of these theoretical objections, many competent observers believe that the opsonic index is of

<sup>1</sup> *Biometrika*. Cambridge, 1909, 6, Part iv.

<sup>2</sup> *Proc. Roy. Soc. Med.*, 1908, Med., i. 195.

considerable value in indicating the general trend of the process of immunization, and of even greater value in indicating which of several associated bacteria is the species actually responsible for a condition of disease. Moreover, there can be no doubt that the study of the phagocytic properties of the serum has not only opened up a most interesting chapter in pathology, but has had at least one very important and most beneficial result, in that it has led to a great reduction in both the frequency and the size of the doses of tuberculin generally employed (*see* pp. 333, 335). The effects of minute doses are now recognized, and a great step towards a rational use of the remedy has been taken.

#### COMPLEMENT-FIXATION

The utilization of complement in a bacteriolytic system was originally suggested by Bordet and Gengou as an aid to the identification of bacteria, or conversely for the demonstration of specific antibodies, in the serum of immunized animals. It was afterwards employed in the clinical diagnosis of bacterial infections, and at present finds its chief application in the diagnosis of syphilis under the name of Wassermann's reaction (p. 408). The test depends upon the fact that the same complement is capable of entering into combination with more than one "couple" containing antigen and its specific copula; but complement thus added to one such "couple" is used up or fixed, and is therefore unable to complete a second "couple"—say hæmolysin-erythrocyte, if such is subsequently added to the mixture—and hæmolysis fails to take place. If, on the other hand, specific copula is absent from the serum employed in the first mixture, the complement remains free to enter into combination with the hæmolysin-erythrocyte couple—and hæmolysis takes place.

In the diagnosis of bacterial infections an emulsion of the bacterium suspected to be the cause of the disease serves as the antigen, and the patient's serum is added on the assumption that it contains the specific copula; to

these two reagents serum containing complement is added, and the mixture incubated for an hour at body temperature. A "couple" consisting of mammalian red blood-cells and the corresponding hæmolysin is then added, and the incubation repeated, when the presence or absence of hæmolysis serves to indicate the absence or presence of the specific copula in the patient's serum. The test as applied to the diagnosis of syphilis is discussed further in Chapter XXI.

#### DIAGNOSIS BY PRECIPITIN REACTION

**Wassermann-Uhlenhuth test for blood.**—Tchistowitsch<sup>1</sup> injected rabbits with the serum of horses, and found that the rabbits' serum as a result of the injections acquired the power of precipitating part of the albumin of the horse-serum when mixed with it. Other observers amplified these results, and in consequence Wassermann proposed to use serum from animals previously injected with human serum to distinguish human from other blood. Uhlenhuth<sup>2</sup> tested nineteen kinds of blood, and found that with such a serum human blood alone gave the reaction. Stern,<sup>3</sup> however, showed that monkey's blood gave a reaction similar to that produced by human blood.

In order to prepare the antiserum a rabbit is first injected with sterile freshly defibrinated blood, or preferably with sterile serum, at intervals of four or five days for a period of two or three weeks. The animal is bled from a vein, and, when the clot has separated, the serum is pipetted off and stored in a cool place.

In performing the test the suspected blood is mixed with a small quantity of normal saline solution and filtered: the filtrate is divided between two test-tubes, to one of which is added twice the volume of antiserum. In another tube is placed blood from some other mammal, together with

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1899, xiii. 406.

<sup>2</sup> *Berl. klin. Woch.*, 1901, p. 187.

<sup>3</sup> *Deut. med. Woch.*, 1901, p. 135.

antiserum; and in a fourth tube antiserum mixed with normal saline solution. All four tubes are incubated at 37°C. for one hour, then allowed to stand at room-temperature for four hours. If the suspected blood is of human origin the first tube alone will show evidence of precipitation, the remaining three control tubes being perfectly clear. The test is active even in extreme dilution—Stern quotes a positive reaction with blood diluted 50,000 times.

### TOXINS AS MEANS OF DIAGNOSIS

In some diseases the injection into the affected animal or patient of the toxins of the bacillus causing the condition produces a febrile reaction, and use has been made of this as a means of diagnosis in the case of glanders and tuberculosis. The preparation used for the diagnosis of the former disease is known as "mallein," and is much used in veterinary practice to discover the existence of the disease in horses, in which it is often very latent. Tuberculin is similarly used on cattle to reveal the existence of tuberculosis, and has also been employed in human patients, though it has not come into general use, partly owing to the disagreeable nature of the effects produced, and to a real or supposed risk of doing harm to the sufferer; partly from the existence of other means of diagnosis, such as physical examination, and the search for bacilli in the expectoration. It is, however, used in the percutaneous, the cutaneous, and the ophthalmic reactions (*see* p. 307), and in suitable instances by subcutaneous injection

### PHYSICO-CHEMICAL METHODS

Brief allusion may be made to certain methods of diagnosis which depend on alterations in the physical and chemical qualities of blood-serum in special diseases, or in relation to the reactions of immunity. For details the original papers should be consulted.

The combination of antigen and antibody, with absorption of complement, is said to be accompanied by an



alteration in the surface-tension of the fluid in which the reaction takes place. Observations were made on the phenomenon by Weichardt and by Ascoli, and the latter devised a special test known as the **meiostagmin reaction**,<sup>1</sup> in which the number of drops formed by a known quantity of a mixture of serum, antigen, and antibody is counted, and compared with that formed by a similar mixture made with normal serum as a control. The test is positive if the drops are smaller, and are therefore more numerous, with the serum to be tested. The instrument used for counting them is Traube's stalagmometer.

Allied to this reaction is the so-called **epiphanin**<sup>2</sup> reaction of Weichardt, which depends on an alteration in the reaction to phenolphthalein of an exactly neutralized mixture of barium hydrate and sulphuric acid in the presence of an antigen-antibody combination. For the performance of the test 0.1 c.c. of a 1:10 dilution of serum in normal saline solution is mixed with 0.1 c.c. alcoholic extract of syphilitic foetal liver (also diluted 1:10). One cubic centimetre of decinormal sulphuric acid is added, and then an amount of barium hydrate solution which has been previously found to neutralize exactly the above amount of the dilute acid. On adding a drop of alcoholic solution of phenolphthalein, a red colour appears if the serum tested is syphilitic: a control test with normal serum remains colourless. The test is said to give good results as a diagnostic agent in syphilis (Seiffert),<sup>3</sup> but it has not come into general use.

Alterations in the quantities of globulin present in the serum are the basis of the reactions named after Porges and Hermann and Perutz. To elicit **Porges' reaction**<sup>4</sup>

<sup>1</sup> From μέλον, smaller, and στάζω, I drip—i.e. smaller drops. See Ascoli, *Münch. med. Woch.*, 1910, p. 62.

<sup>2</sup> ἐπιφάνεια, surface. Weichardt, *Zeitschr. f. Immunitätsforsch.*, 1910, Orig., vi. 842.

<sup>3</sup> *Deut. med. Woch.*, 1910, p. 2333.

<sup>4</sup> De la Motte, *ibid.*, 1910, p. 1561.

0.2 c.c. of clear sterile inactivated serum is mixed with the same quantity of a 1 per cent. solution of sodium glycocholate, and the mixture is allowed to stand at room-temperature for sixteen to twenty hours. At the end of that time, if the test is positive, definite floccules have formed in the mixture, and tend to rise to the surface of the fluid. The method of performing the **Hermann-Perutz**<sup>1</sup> test is very similar.

In **Rivalta's reaction**<sup>2</sup> two solutions are prepared, the first consisting of a drop of saturated solution of sodium carbonate in 100 c.c. of distilled water, and the second of 2 drops of glacial acetic acid in 100 c.c. of water. The blood or serum to be tested is diluted 1 : 100 with the first fluid, and then a drop of this mixture is taken up on a glass rod and allowed to fall into some of the second fluid in a beaker. If the test is positive, a white ring appears where the drop falls, probably consisting of globulin.

**Klausner's reaction**,<sup>3</sup> consisting in the formation of a cloudy precipitate on adding distilled water to syphilitic serum, is said to depend on the presence of a lipoid body.

<sup>1</sup> *Med. Klinik*, 1911, No. 2.

<sup>2</sup> *Polieclinico*, 1905, vol. xii. ; also 1910, *Sez. Prat.*, Nos. 22 and 23. Cf. Gironi, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv. 229.

<sup>3</sup> *Biochemisch. Zeitschr.*, 1912, xlvii. 36.

## CHAPTER V

### DIPHTHERIA

**Nature of diphtheria.**—Diphtheria, derived from the Greek word *διφθέρα*, a skin or piece of leather, was a term originally applied to cases of sore-throat characterized by the presence of "false membrane." When the condition came to be examined bacteriologically, it was found that the great majority of these cases are associated with the growth of a particular bacillus (*B. diphtheriae*). It was therefore assumed that all cases of the disease were due to this organism, and it became necessary from the public-health point of view to diagnose diphtheria solely on bacteriological findings. A case of sore throat in which the bacillus is found is now called "diphtheria," apart from the presence or absence of the characteristic clinical symptoms (membrane-formation),<sup>1</sup> while cases of membranous sore-throat in which pneumococci, pneumobacilli, or streptococci are alone present, and in which no diphtheria bacilli can be detected, are not regarded as instances of the disease. The practical result is to change the connotation of the term diphtheria from that of "membranous sore-throat" to that of "sore-throat due to *B. diphtheriae*." A recognition of these facts will be seen to be of importance when the evidence for the value of antitoxic serum is discussed.

**Causal organism.**—The *Bacillus diphtheriae* was first discovered by Klebs in the year 1883, and was cultivated

<sup>1</sup> At the hospitals of the Metropolitan Asylums Board cases are now classified as "diphtheria" and "bacteriological diphtheria," the latter, in which there are no clinical signs of diphtheria, being a non-fatal condition, although, as "carriers," individuals harbouring diphtheria bacilli are a source of danger to the community.

by Loeffler in the following year; hence it is generally known as the Klebs-Loeffler bacillus. It belongs to a group of organisms the exact relations between the members of which are not definitely decided. The most closely allied form is the so-called pseudo-diphtheria bacillus, which resembles the pathogenic organism, but is not virulent for animals. Its relation to cases of sore-throat in human beings is still undecided. Culturally it is quite distinct from the Klebs-Loeffler bacillus; moreover, it is said not to be agglutinated by the serum of animals rendered immune against the Klebs-Loeffler bacillus—strong evidence of the diversity of the two organisms. Another closely allied, if not identical, organism is the *Bacillus xerosis*, which is met with in the conjunctival sac and was at one time supposed to be causally associated with the affection of the eye known as xerosis conjunctivæ; it is also of common occurrence in the discharge of otitis media, in association with the pathogenic organisms responsible for the suppuration, and is a very common saprophyte of the external genitals. Diphtheroid bacilli have also been found in the disease called noma or cancerum oris.

**Occurrence in the body.**—The Klebs-Loeffler bacillus is met with not only in cases of diphtheria, but also in chronic nasal discharges. It may likewise be found in a virulent condition in the throats of healthy persons, and may gain a footing on any open wound and there give rise to the formation of false membrane.

In cases of membranous sore-throat in which the diphtheria bacillus is found, it may occur either in almost pure culture or mixed with other organisms, especially streptococci. These mixed cases are generally more severe, and the prognosis is worse than in simple diphtherial infection. As in the throat, so also on wounded surfaces, other bacteria, such as streptococci and the pneumobacillus of Friedländer, may form false membrane, so that every such formation is not diphtheritic in the bacteriological sense of the word, i.e. caused by the *B. diphtherie*.

In cases of diphtheria the bacilli remain for the most part confined to the false membrane in the fauces; no general infection of the blood takes place as a rule, though in severe cases some of the bacilli may gain access to the blood-stream; apparently, however, they do not multiply therein, and consequently do not give rise to a true septicæmia. By direct extension, however, as can frequently be demonstrated at the necropsy, the diphtheria bacillus may invade the bronchi and so give rise to a membranous bronchio-pneumonia; and in one instance the writers have seen the extension of the membrane into the stomach.

Diphtheria bacilli manufacture a substance, probably in the nature of a ferment, which is absorbed and carried by the blood and lymph all over the body. This ferment, by its action on the tissues, gives rise to other poisonous materials or secondary toxins. The bodies of the bacilli themselves are not so poisonous as their soluble products; thus Kossel<sup>1</sup> showed that if the actual bacteria were washed free from the poison and then killed, the dead bodies had very little toxic influence when injected into animals.

**Toxins of diphtheria.**—The nature of the poisons manufactured by the diphtheria bacillus was studied very early in the history of modern bacteriology, since the organisms form soluble toxins which can be readily obtained in culture-media.

Roux and Yersin<sup>2</sup> were the first who discovered the presence of diphtherial toxins in peptone-broth cultures of the bacilli (1888, 1889). Solutions of the poisons may be prepared by growing the organisms in broth for periods of two to four weeks, and then either passing the fluid through a porcelain filter, so as to strain off the bacilli, or adding a germicide of some sort to it, so as to kill them. Toluol has been used for the latter purpose by Ehrlich and

<sup>1</sup> *Centralbl. f. Bakt.*, I., 1896, xix. 977.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1888-9.

Wassermann.<sup>1</sup> The fluid is well shaken up with this substance, which separates, on standing, into a layer floating on the surface of the broth. In this condition the preparation can be kept indefinitely, as the toluol prevents any decomposition taking place. The bodies of the bacilli sink to the bottom of the flask. A special method of growing the bacteria was devised by Aronson,<sup>2</sup> in which they are induced to form a scum or coat on the surface of the broth; thus treated, they produce a much stronger toxin than when they are cultivated in the ordinary way, diffused through the fluid medium.

The effects of the poison are seen equally well whether the living bacilli or the prepared toxins are used for experimental injection. A guineapig which has received a dose of the organisms subcutaneously presents first at the site of injection an œdematous swelling; this is followed by enlargement of the neighbouring lymphatic glands. The animal appears to become weaker and weaker, and dies, if a moderately strong dose has been given, in the space of about four days. An examination of the body then shows the existence of œdema and hæmorrhage at the site of injection, and serous effusion into the cavities of the pleuræ, pericardium, and peritoneum. The bacilli are not found to have become generalized throughout the body. Very large doses of toxins or very virulent bacilli may produce death in twenty-four hours.

If weaker doses of poison are administered, insufficient to cause death, the most marked phenomenon may be the local swelling; and if life is prolonged for as much as a fortnight, paralytic symptoms may supervene, and the guineapigs die of asthenia. There is reason to believe that at least three separate poisons are manufactured by the Klebs-Loeffler bacillus: one, which is the most important, causes death by a general neuro-muscular intoxication; a second produces the local œdema at the point of inoculation,

<sup>1</sup> *Zeitschr. f. Hygiene*, Bd. xix., 1893.

<sup>2</sup> *Berlin. klin. Woch.*, 1894, p. 426.

which may actually go on to necrosis of the superficial tissues; and the third is responsible for the paralysis which sometimes occurs as a sequel.

As to the exact chemical composition of the toxins, little is definitely known. Roux and Yersin considered that the main poison was of the nature of a ferment; they found that the toxic substance which they succeeded in isolating did not act in the presence of acid. Sidney Martin was also led to believe that the primary poison is a ferment. He isolated from the tissues of animals dead of the disease, as well as from the culture-media in which the organisms had been grown, a series of albumoses (proto-, deuter-, and hetero-albumose), as well as an organic acid. He considered that the albumoses were formed in the tissues, especially in the spleen, not in the false membrane. In this latter the ferment was generated, and thence it was absorbed by the blood-vessels. Brieger and Bör<sup>1</sup> grew the bacilli in dialysed urine, a non-albuminous fluid, and precipitated the toxin by means of zinc chloride. The material thus prepared was non-albuminous; it was very sensitive to oxidizing agents, but resistant to reducing substances. It was highly toxic to animals, and the injection of it in small quantities produced immunizing substances in their serum. These observers found that the bodies of the bacteria contained a substance which was capable of causing necrosis of living tissues, and which did not give rise to antitoxin in the serum. In this respect they are at issue with Kossel,<sup>2</sup> who found the bodies of the bacteria only slightly toxic.

#### DIPHTHERIAL ANTITOXIN

**Manufacture of antitoxin.**—For the production of antitoxin it is necessary to prepare a toxin of the highest possible virulence. Certain strains of the bacillus appear to be specially adapted to form toxins in artificial media,

<sup>1</sup> *Deut. med. Woch.*, 1896, p. 784.

<sup>2</sup> *Loc. cit.*, p. 96.



adopting the peculiar form of growth already described (formation of a pellicle on the surface of the nutrient fluid), which is found to be most advantageous for this purpose. When the organisms have grown for about a fortnight on the culture-fluid, the latter is passed through a porcelain filter; the bacilli are thus removed, and the filtrate is ready for use.

The horse selected for the production of antitoxic serum is submitted to a preliminary examination with malleïn and tuberculin to ensure that it is free from glanders and tuberculosis. If it fails to react to these tests, it receives an injection of a small quantity of the toxin<sup>1</sup> ( $\frac{1}{2}$  to 1 c.c.) subcutaneously in the loose tissue at the root of the neck. The injection is followed by considerable local reaction, causing the appearance of a large swelling, while the horse exhibits signs of fever and constitutional disturbance. It is necessary to wait till these symptoms have subsided before administering a second injection, which may be given on the opposite side of the neck. The doses are gradually increased till as much as an entire litre of the toxin may be injected for a single dose. The febrile disturbance produced by the poison becomes less and less as the treatment continues. It appears to be a good sign that the horse should react strongly at first, as such animals seem to produce in the end a more highly antitoxic serum. Some horses fail altogether to form antitoxin; probably the receptors of their cells have not enough affinity for the toxin, and so the number killed is not sufficient to stimulate reproduction in excess.

The injection of each dose of poison is followed by an immediate fall in the antitoxic value of the serum of the horse, but this rises again in the course of a day or two to a point higher each time than that at which it previously stood. It is important not to give a fresh dose of toxin till this rise in antitoxic power has taken place; otherwise the antitoxin present may actually be diminished instead of increasing. As a rule the injections are given

<sup>1</sup> I.e. the toxic culture-fluid.

about once in three to seven days. The antitoxic power of the blood reaches its maximum in about six months. A horse will not go on indefinitely producing antitoxin; its power in this direction appears to become exhausted after a time.

**Standardization of toxin and antitoxin.**—As has already been pointed out, it is not possible to weigh or measure toxins and antitoxins as we do ordinary drugs, and therefore their strength can only be measured by means of physiological tests, that is to say, by determining experimentally the effects produced on living animals. For the purpose of standardizing the toxins of diphtheria, guineapigs are the animals generally used, as it is found that they react in a very constant manner to the poison; those of the same weight being killed in approximately the same period of time by equal doses of a given toxin. A unit dose of toxin is that amount of any preparation of diphtherial poison which will just suffice to kill a guineapig weighing 250 grm. in a period of four days. This is also known as the “minimal lethal dose (m. l. d.).”

A *unit of antitoxin* is the smallest quantity which, being mixed with 100 minimum lethal doses<sup>1</sup> of toxin and injected into a guineapig, prevents the appearance of any toxic symptoms.

This method of standardization is the one inaugurated by Ehrlich. It was necessary in the first instance to establish a toxic unit, and then to calculate from this the antitoxic unit. When this had once been done, however, it became easier subsequently to calculate backwards from antitoxin to toxin, since the former is more easily preserved, not varying in strength even when kept for considerable periods of time. A standard antitoxin is supplied by the Serumprüfungs Institut at Frankfort-on-Main, and this standard is now universally adopted.

<sup>1</sup> This quantity of toxin, sufficient to kill 100 guineapigs, and exactly neutralized by one unit of antitoxin, is called by Ehrlich the  $L_0$  dose.

A method of standardizing antitoxin, founded on the determination of the L + dose (*see* p. 102), has recently been introduced in place of the one described above. A standard antitoxin being available, unit doses of it are taken; varying quantities of (any) toxin are added to these, and the mixtures are injected into guineapigs, until the exact mixture (1 unit antitoxin and  $x$  toxin) necessary to produce death on the fourth day is discovered. This amount ( $x$ ) of the toxin is then mixed with varying quantities of the antitoxin which is to be standardized, and the quantity of this latter which must be added to the above ( $x$ ) amount of toxin, in order that the animal may be killed in the given time, is ascertained. This quantity is thus proved to contain exactly one unit of antitoxin, its action being precisely equivalent to that of the original standard unit.

**Interaction of toxin and antitoxin.**—From the facts just recorded it has been assumed that the relation between given specimens of toxin and antitoxin is constant, the same quantity of the latter being always required to neutralize exactly a given amount of the former. This is practically true within limits. The interaction between the two substances therefore resembles a simple chemical combination, similar to that which takes place between an acid and an alkali. But in the case of the substances which we are considering certain curious phenomena have been observed, showing that we are not dealing with a case of simple chemical combination. If we take a certain quantity of a simple acid and add to it the amount of the alkali which exactly neutralizes it, we have a mixture corresponding with the mixture of one unit of antitoxin with 100 minimal lethal doses of toxin. If to the former mixture we add any fresh quantity of the acid, it will remain uncombined and capable of producing its normal effects (combining with more alkali, etc.). If, however, we take the mixture of toxin and antitoxin, and add to it one minimal fatal dose of toxin, we do not find that this additional toxin has still its usual effect, viz. to kill a guinea-

pig of 250 grm. in four days. On the contrary, if the mixture (unit of antitoxin + 100 minimal fatal doses of toxin + 1 extra minimal fatal dose) is injected into a guinea-pig, the animal recovers from the injection, only exhibiting a certain amount of œdema at the point of injection. If still further quantities of toxin are added, it will be found that quite a large number of toxic units must be added before a point is reached at which the animal dies in four days. This additional quantity is called by Ehrlich the L+ dose.

We may make the same experiment in another manner. If we take the amount of toxin which is exactly neutralized by one unit of antitoxin, viz. 100 lethal doses, add to it  $\frac{1}{2} \frac{0}{0} \frac{0}{0}$  of a unit of antitoxin, and inject the mixture into a guinea-pig, the animal does not die, but only suffers from some local œdema. This might, indeed, have been foretold, as there should theoretically be set free only one-half of a minimal lethal dose of poison. If, however, we proceed further in the same way, and add to the same quantity of toxin  $\frac{1}{2} \frac{0}{0} \frac{8}{0}$  of a unit of antitoxin, we should expect death to occur on the fourth day, as one lethal dose should now be available. But again only local œdema results. Proceeding in this way, it is found that, even if  $\frac{1}{2} \frac{5}{0} \frac{0}{0}$  of a unit is added, the mixture is still incapable of killing the animal in the stated time. When, however, the 100 lethal doses of toxin are mixed with only  $\frac{1}{2} \frac{1}{0} \frac{0}{0}$  of a unit of antitoxin, then one minimal lethal dose is set free and the usual effect is produced. It is found at this point that for each  $\frac{1}{2} \frac{1}{0} \frac{0}{0}$  of a unit of antitoxin that is subtracted, one lethal dose is set free. This continues till a point is reached at which we have arrived at a mixture of 100 m. l. d. of toxin with  $\frac{5}{2} \frac{0}{0} \frac{0}{0}$  of a unit of antitoxin; this is capable of killing 100 guinea-pigs. Any further diminution of antitoxin is without effect.

Put in other words, it appears that it is possible to add to 100 lethal doses of poison as much as one-quarter of the total amount of antitoxin which will exactly neutralize them, without decreasing the available toxic capacity. If a

further half-unit of antitoxin is added, the whole of the poison is neutralized.

The explanation of these phenomena given by Ehrlich is that the crude poison, if it may so be called—the culture-medium in which the bacteria have grown—contains several different substances, all of which have the power of combining with antitoxin. They have, however, different degrees of affinity for the latter. The body which has the

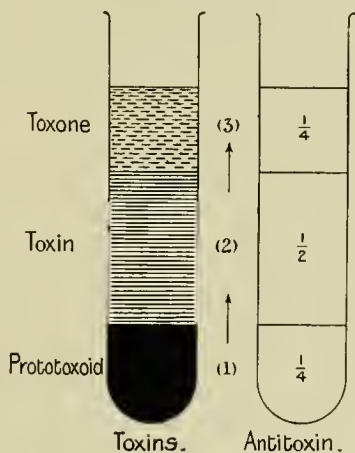


Fig. 17.—Diagram illustrating the process of saturation of diphtherial toxin with antitoxin.

greatest avidity for antitoxin is called “prototoxoid.”<sup>1</sup> The main toxin, which causes the death of the guineapig, occupies an intermediate place in point of affinity, while a third substance, called “toxone,” has the least affinity of all. This last appears to be the body which is responsible for the local œdema seen at the point of injection of crude diphtherial toxins.

The accompanying diagram (Fig. 17) will perhaps serve

<sup>1</sup> It has been suggested that toxoids consist of free “haptophore” molecules of toxin which have lost their “toxophore” element (*see* p. 25).

to make a little clearer what happens on gradually adding antitoxin to toxin. The tube on the left shows the relative proportions of each substance present in a specimen of crude poison. If now antitoxin be added, filling up, as it were, the tube from the bottom, it will first of all neutralize the prototoxoid, one-quarter of the whole antitoxin being thus occupied. The next two quarters will be taken up by the toxin, and the last quarter of all by the toxone. The first addition of antitoxin does not reduce the toxicity of the mixed poisons, because it merely neutralizes the prototoxoid, which has no poisonous properties. The second addition counteracts the most active poison, the true toxin; while the last addition prevents the local effects which are caused by the toxone.

Again, if we take a mixture in which the toxins are exactly neutralized by antitoxin (neglecting for the sake of simplicity the prototoxoid), the addition of a further unit of toxin will tend to set free an equivalent quantity of toxone, which has less affinity for the antitoxin; and on adding still further quantities of toxin a fatal amount will not be reached till all the toxone has been set free and its proportion of the antitoxin annexed by the toxin.

The following illustration of the interaction of antitoxin and toxin in diphtheria, by means of an analogous process in ordinary chemistry, is given by Emery.<sup>1</sup>

“ You remember that in estimating chlorides by titration with silver nitrate you add a little chromate of potash to the solution to be tested. The silver has a greater affinity for the chloride than for the chromate, and you get a white precipitate of silver chloride until all the soluble chlorides have been decomposed, and then you begin to get a chocolate-coloured precipitate of silver chromate. In precisely the same way, when you add antitoxin to diphtheria poison, the first portion added goes to combine with the prototoxoids, and these must be completely saturated before any toxin is neutralized.”

<sup>1</sup> *St. Bartholomew's Hosp. Journ.*, Dec., 1902, p. 37.

In addition to the above facts, there are certain peculiarities about the mixture resulting from addition of antitoxin to toxin which throw doubt on the explanation of their interaction as a simple chemical combination. Thus, a mixture of toxin and antitoxin may be made which is neutral for a mouse, but which when injected into a guineapig may cause toxic symptoms; and Behring<sup>1</sup> found that a mixture of 1 unit of toxin with a corresponding amount of antitoxin, which is neutral for a guineapig, is still toxic for the ass, and that apes are killed by two or three injections of a mixture of 1 unit of toxin with 40 units of antitoxin. Again, if the mixture be heated to 100° C., the antitoxin is destroyed and the toxin remains unneutralized. Similarly, if the mixture be passed through a porcelain filter, the toxin passes through in the filtrate, and the antitoxin remains behind; while if the same mixture be injected into an animal, the toxin may be eliminated in an active condition in the urine. It seems difficult to explain these phenomena on the basis of a simple chemical combination. Some authorities have maintained that antitoxin does not act directly on the toxin, but indirectly through the medium of the living cell, which it stimulates in some way to resist the poison (Roux, Büchner). Danysz<sup>2</sup> considers that toxin and antitoxin may combine in different proportions to form a series of "compounds," somewhat analogous to the series of oxides of nitrogen,  $N_2O$ ,  $N_2O_2$ ,  $N_2O_3$ , etc.; and Bordet,<sup>3</sup> who also holds this view, considers that toxone is in reality a molecule of toxin incompletely saturated with antitoxin. The question is a very difficult one and cannot be decided on the data at present available. It seems probable that a definite chemical combination occurs, but that the affinity between the two substances is comparatively slight, so that the combination only takes place slowly and is readily decomposed. Arrhenius and Madsen

<sup>1</sup> *Deut. med. Woch.*, 1913, p. 873.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 345.

<sup>3</sup> *Ibid.*, 1903, xvii. 185.



hold this view and consider that the results ascribed by Ehrlich to the action of toxone are really due to the presence of dissociation-products.

Von Calcar<sup>1</sup> states that he was able by fractional dialysis to separate the toxin from the toxone, thus proving them to be distinct chemical bodies, but his methods and results have been called in question by Römer.<sup>2</sup>

In the case of the toxin and antitoxin of tetanus, Behring<sup>3</sup> considers that a third body, which he calls the "conductor," is necessary to bring about combination, this body acting in the same manner as the copula in hæmolysis. If this is proved to be the case in tetanus, it is almost certain that the interaction of other toxins and antitoxins will be found to take place in the same manner. A somewhat similar view is propounded by Bordet and Gay<sup>4</sup> in the case of diphtherial toxin and antitoxin.

Some interesting experiments by Dönitz<sup>5</sup> throw light on the action of toxin and antitoxin within the body of a living animal. This observer found that if a dose of toxin was injected into a rabbit, it would be neutralized by the same amount of antitoxin which would neutralize it *in vitro*, provided that the latter were given within a space of nine minutes. If more than this time had elapsed, it was necessary to administer a considerably larger dose of the antitoxin; but if this larger quantity were given, it was still possible to prevent the appearance of any serious ill effects. If, however, a space of time exceeding about two hours were allowed to pass after the toxin had been given and before the antitoxin was injected, no amount of the latter would suffice to avert a fatal issue. It would appear from this that we can distinguish three separate periods corresponding with distinct stages in the process of intoxi-

<sup>1</sup> *Berlin. klin. Woch.*, 1905, xli. 1368.

<sup>2</sup> *Beitr. z. exper. Therap.*, 1905, Hft. 18-41.

<sup>3</sup> *Deut. med. Woch.*, Aug. 27, 1903.

<sup>4</sup> *Ann. de l'Inst. Pasteur*, 1906, xx. 467.

<sup>5</sup> *Arch. Internat. de Pharmacodyn.*, Bd. 5, 1899.

cation: (1) At first the poison is circulating in the blood, and has not yet attacked the cells. (2) Later on it has entered into some sort of combination with them, but this is so loose that the presence of a large quantity of antitoxin is capable of withdrawing the toxin from them again. (3) The toxin has become so firmly fixed to the cells that no amount of the remedy is capable of undoing the combination. What constitutes the difference between the last two stages is unknown. Perhaps in the former of the two the toxin has only attached itself to the side-chains, whereas in the latter it has entered into combination with the whole body of the cell-protoplasm.

The experiments just recorded point to the necessity for the early administration of antitoxin when used as a remedy for diphtheria. It is important to give it before the poison has gained so firm a hold upon the cells that it can no longer be withdrawn. It is fortunate that diphtheria is a disease in which it is possible to recognize the existence of infection at a comparatively early stage, since the false membrane appears on the fauces some time before any profound intoxication of the entire system has taken place. To this fact is due, no doubt, the infinitely greater success that has attended the use of diphtherial antitoxin as compared with that prepared for tetanus. In the latter disease the existence of the infection is only recognized by the appearance of the symptoms of general intoxication. It is then, in many cases, already too late to hope for good results from the administration of antitoxin. Statistics are given later (p. 126) which afford incontrovertible clinical evidence of the value of early injection of diphtherial antitoxin.

**Strength of antitoxic serum.**—For human use it is important to possess a serum containing a large number of antitoxic units in a small volume, since it is not desirable to inject a larger quantity of the fluid than is absolutely necessary. Not only does the injection of a large dose cause a considerable local swelling, which is only slowly absorbed, but the various unpleasant effects which

at times follow an injection are dependent to a great extent on the actual volume of the serum which is administered. The majority of serums on the market contain 300 to 500 units in each cubic centimetre. Hence it is not often necessary to give more than 10 c.c. for a dose. Stronger specimens can be obtained at a higher price. Diphtherial antitoxin is generally supplied in liquid form, made up with a little antiseptic as a preservative. It can also be obtained in the desiccated form. According to Chiadini<sup>1</sup> it appears to keep well for a period of at least eighteen months; after two years it begins to deteriorate a little, but still possesses considerable antitoxic power; after four years it is valueless. Ordinary degrees of light and heat do not affect its potency, nor does the addition of antiseptic agents. MacConkey states that it loses strength six times as quickly at 36° C. as at 0° C.<sup>2</sup>

**Refined antitoxin.**—Gibson<sup>3</sup> endeavoured to separate the antitoxin from the other constituents of the serum, by precipitation of the pseudo-globulin, which was redissolved and named "refined antitoxin." This preparation is said to retain the full antitoxic value of the original serum, and to be equally useful in the treatment of diphtheria. It also keeps well (Park and Throne). A very similar preparation has been introduced by Brieger and Krause.<sup>4</sup>

**Value of antitoxin.**—It is extremely difficult to obtain definite proof of the curative value of any drug, since the course of almost every disease is variable, and sudden improvements and relapses are liable to occur from natural causes, apart from the action of any remedy. The fluctuations are often ascribed to any drug which is being administered at the time, and there is no means by which the question, *Post hoc* or *Propter hoc*? can be decided. In the

<sup>1</sup> *Gaz. degli Ospedali*, 1902, No. 60.

<sup>2</sup> *Journ. of Hygiene*, 1912, xii. 511

<sup>3</sup> *Journ. Biol. Chem.*, 1906, i. 161.

<sup>4</sup> *Berl. klin. Woch.*, 1907, p. 946.

case of diphtheria the natural variations of the disease are even more marked than in many other disorders, and it is impossible to judge of the efficacy of antitoxin with any approach to accuracy in individual cases.

Dependence must therefore be placed to a great extent on collected statistics. Even here a manifest source of fallacy is introduced by the undoubted fact that infective diseases exhibit great fluctuations in virulence when viewed over considerable periods of time, the mortality from them rising and falling in accordance with obscure periodic laws which are not well understood. Hence a fall in the mortality of an infective disease may occur apart from any new remedy which has come into vogue during the period of time under consideration. In the case of diphtheria there is reason to believe that the disease has become more common in recent years, and also that the type of case seen is, on the whole, less virulent<sup>1</sup>—apart from the use of antitoxin—than used to be the case. It does not seem, therefore, to be logical to ascribe to this remedy all the reduction which has undoubtedly taken place in the mortality from diphtheria. We have to remember also that, as previously stated, there is a tendency to class as diphtheria, owing to the mere presence of *B. diphtherie* in the throat, cases which in earlier days would not have been considered to be suffering from this disease (e.g. cases of mild sore-throat without any formation of membrane, which would in all probability recover without any treatment); such instances swell the number of cases of diphtheria without adding to the deaths which occur, thus reducing the rate of mortality. All these facts must be taken into account when we endeavour to form a scientific judgment as to the interpretation to be placed upon the available statistics with regard to the influence of antitoxin on the course of diphtheria. With this preliminary caution we may proceed to consider the figures actually given by different authorities.

<sup>1</sup> This seems to occur with all infective diseases—as they become more widely spread, so they diminish in virulence.

A very instructive table is to be found in the Reports of the Metropolitan Asylums Board, giving the total number of admissions of cases suffering from each of the notifiable diseases, and the mortality which occurred in the Board's hospitals and throughout the country in each class. From it we extract the following data with regard to diphtheria.

From the table (p. 111) we see that a very marked diminution has occurred in the case-mortality in the hospitals under the Metropolitan Asylums Board since the use of antitoxin became general. Reasons have already been given for thinking that not all of this apparent diminution can be rightly attributed to the new remedy, and if these statistics stood by themselves some doubt might still exist as to the value of antitoxin. But these figures are confirmed by reference to those obtainable from other parts of the country and of the world. Almost everywhere the mortality from diphtheria seems to have fallen at about the same time, and this simultaneous effect can hardly be entirely a coincidence.

It is noteworthy that, while the case-mortality in these hospitals has so distinctly fallen, as shown by the table, yet the mortality throughout the country generally did not decrease up to 1901. Since that date both the general and the hospital mortality have fallen notably. This decline cannot, however, be altogether attributed to the use of serum, for the general mortality for the years 1871-6 was only 0.11; that for the following six years, 0.15; and that for the next five years, 0.23. There is clearly a rise and fall in the mortality apart from the use of any particular remedy.

To show that the fall in diphtheria-mortality has been general throughout the world and not confined to any one place, it may be worth while to quote statistics derived from a variety of sources. Speaking of New York, Billings<sup>1</sup> states that since the introduction of antitoxin a steady fall

<sup>1</sup> *New York Med. Journ.*, Feb. 17, 1900.

# DIPHTHERIA STATISTICS

111

TABLE <sup>1</sup> SHOWING ADMISSIONS FOR DIPHTHERIA TO METROPOLITAN ASYLUMS BOARD HOSPITALS FOR THE YEARS 1888-1913, WITH MORTALITY-RATE IN THESE HOSPITALS AND THROUGHOUT THE COUNTRY

Year	Admissions	Deaths	Percentage mortality in hospital	Annual mortality per 1,000 estimated population
1888	99 <sup>2</sup>	46 <sup>2</sup>	59·35 <sup>2</sup>	0·32
1889	722	275	40·74	0·39
1890	942	316	33·55	0·33
1891	1,312	397	30·63	0·32
1892	2,009	583	29·35	0·46
1893	2,848	865	30·42	0·76
			Average of 5 years, 33 per cent. (about).	Average of 6 years, 0·43 per cent.
1894 <sup>3</sup>	3,666	1,035	29·29	0·62
1895	3,635	820	22·85	0·54
1896	4,508	948	21·20	0·60
1897	5,673	987	17·69	0·51
1898	6,566	991	15·37	0·39
1899	8,676	1,182	13·95	0·43
1900	7,873	988	12·27	0·34
1901	7,622	849	11·15	0·29
			Average of 7 years, about 16 per cent.	Average of 7 years, 0·44 per cent.
1902	6,520	739	11·0	0·25
1903	5,072	504	9·7	0·16
1904	4,687	469	10·0	0·16
1905	4,148	347	8·3	0·12
1906	5,218	445	8·8	0·15
1907	5,744	544	9·6	0·16
			Average of 6 years, 9·5 per cent.	Average of 6 years, 0·16 per cent.
1908	5,230	507	9·7	0·15
1909	4,393	432	9·4	0·13
1910	3,634	281	7·8	0·09
1911	5,034	428	8·4	0·14
1912	4,844	331	6·2	0·10
1913	5,076	330	6·2	0·09
			Average of 6 years, 7·9 per cent.	Average of 6 years, 0·11 per cent.

<sup>1</sup> Extracted from the Report of the Statistical Committee of the Metropolitan Asylums Board. *Annual Report*, 1913, p. 154.

<sup>2</sup> Number of cases too small to be of value. These figures are therefore neglected in computing averages.

<sup>3</sup> Treatment with antitoxin introduced during this year.

in both the number of cases and the number of deaths took place. He gives the following table, which may be compared with that on p. 111.

MORTALITY IN NEW YORK BEFORE AND AFTER THE INTRODUCTION OF ANTITOXIN (BILLINGS)

Year	Cases	Deaths	Mortality per cent.
1891	5,364	1,970	36·7
1892	5,184	2,196	40·6
1893	7,021	2,558	36·4
1894	9,641	2,870	29·7
1895 <sup>1</sup>	10,353	1,976	19·1
1896 <sup>2</sup>	11,399	1,763	15·4
1897	10,896	1,590	14·6
1898	7,593	923	12·2
1899 <sup>3</sup>	8,240	1,087	13·1

The death-rate per 10,000 inhabitants previous to the advent of antitoxin was, according to Park, from 15 to 18·8. After its introduction it fell to 7, the average number of deaths in New York falling from 2,733 to 1,341 (taking the averages of fifteen years before and four years after antitoxin). In Berlin<sup>4</sup> the average of deaths per 100,000 inhabitants in pre-antitoxin days was 90·6: it fell to 38·5 in the five succeeding years. In Paris the fall was from 62·2 to 13·3. It cannot be maintained, indeed, that all this reduction in mortality was due to antitoxin; sanitary measures probably helped to reduce the death-rate, and the virulence of the disease may have diminished; but the coincidence of a fall all over the world about the time of the introduction of antitoxin is too remarkable to be altogether accidental.

With regard to case-mortality, Rosenthal<sup>5</sup> collected from various sources figures showing that of 183,256 cases

<sup>1</sup> Antitoxin introduced.

<sup>2</sup> Use of antitoxin became general.

<sup>3</sup> We have not been able to find statistics for more recent years.

<sup>4</sup> Cobbett, *Edinburgh Med. Journ.*, 1900, i. 521.

<sup>5</sup> *Med. Press and Circ.*, 1900, ii. 293.



treated before antitoxin was introduced the mortality amounted to 38·4 per cent. Among 132,548 cases after its use became general the mortality was only 14·6. Felix<sup>1</sup> states that in Roumania, before the remedy was known, the mortality of diphtherial cases was from 41 to 63 per cent.; the introduction of antitoxin has reduced the fatality among cases treated with serum to 12 per cent. Jaeger<sup>2</sup> states that in Mülhausen the death-rate was 52 to 55 per cent. in ordinary cases and 65 to 68 per cent. in laryngeal cases in pre-antitoxin days; whereas it fell to 16 to 20 per cent. and 20 to 25 per cent. respectively after the use of antitoxin became general. Similarly for Vienna Siegert<sup>3</sup> states that :

From 1892-4, of 4,894 cases of diphtheria, over 2,000 died; mortality nearly 50 per cent.

From 1895-7, of 4,143 cases of diphtheria, only 817 died; mortality about 25 per cent.

Enough has now been said to show that the diminution in mortality is not confined to any one part of the world. Other evidence in favour of the remedy may be quoted of an even more convincing nature.

When diphtheria affects the *larynx* the cases are generally more severe than those which are confined to the fauces. Goodall<sup>4</sup> gives some figures as to the efficacy of antitoxin in these cases. Before the days of antitoxin, of 3,275 cases of laryngeal diphtheria, 1,008 recovered (33·8 per cent.), giving a mortality of 66·2 per cent. After the introduction of the remedy, of 3,486 cases of the same nature, 2,522 recovered (72·3 per cent.), a mortality of 27·7 per cent. Taking cases of tracheotomy, the pre-antitoxin rate of recovery was under 30 per cent.; after serum-

<sup>1</sup> *Spitalul.*, 1902, No. 5 (Abstr. in *Centralbl. f. inn. Med.*, 1902, p. 799).

<sup>2</sup> *Deut. Arch. f. klin. Med.*, lxxiii.

<sup>3</sup> *Jahrbuch f. Kinderheilk.*, Jan., 1902.

<sup>4</sup> *Brit. Med. Journ.*, 1899, i. 197.

treatment was inaugurated, the percentage of recoveries rose to 63·4 per cent. The improvement is very remarkable. Goodall concludes: "Whereas in the pre-antitoxin days, of 100 tracheotomies you could not expect to save more than 29, you can now expect to save no fewer than 53. . . . I think I am fully justified in claiming for antitoxin the great reduction in mortality among cases of laryngeal diphtheria that these figures reveal."

Park<sup>1</sup> records 802 laryngeal cases with a mortality of 23 per cent., and Piekema<sup>2</sup> 369 cases of tracheotomy or intubation with 28·2 per cent. of deaths. Both these authors ascribe the success met with to the use of antitoxin.

Still more conclusive evidence is afforded by the statistics of the comparative rates of mortality in cases in which the remedy is administered early in the disease, and in those in which a delay of some days has occurred. The reason for this has been already pointed out (p. 46).

The table on p. 115 gives the results obtained by the Collective Investigation Committee of the American Pediatric Society<sup>3</sup> (1896).

The records of the Brook Hospital, under the Metropolitan Asylums Board, show very similar results.<sup>4</sup> Thus in the years 1897-1907 (inclusive) 250 cases were treated on the first day of the disease, without a single death; of 1,513 cases treated on the second day the mortality was 4·29 per cent.; among 1,690 treated on the third day, 11·24 per cent.; among 1,338 treated on the fourth day, 16·89 per cent.; and among 1,765 cases treated on the fifth and subsequent days it was 18·58 per cent.

<sup>1</sup> *Journ. of the Amer. Med. Assoc.*, 1900, April 14, p. 902.

<sup>2</sup> *Inaugural Dissertation*, Utrecht, 1900 (Abstr. in *Centralbl. f. inn. Med.*, 1902, p. 799).

<sup>3</sup> *Journ. of the Amer. Med. Assoc.*, 1896, ii. 27. Cf. Biggs, *Med. News*, 1899, July 22 and 29, pp. 97 and 137; Larkins, *Journ. Amer. Med. Assoc.*, 1899, p. 7; Park, *ibid.*, 1900, April 14, p. 902.

<sup>4</sup> *M.A.B. Rept.*, 1907, p. 165.

MacConkey,<sup>1</sup> the compiler of these statistics, gives similar figures for the years 1912-13, which, taken together, show 69 cases treated on the first day with no deaths; 288 on the second day with 9 deaths, or 3·1 per cent.; 290 on the third day with 25 deaths, or 8·6 per cent.; 218 on the fourth day with 27 deaths, or 12·35 per cent.; and 332 on subsequent days with 27 deaths, or 8·1 per cent.—this last smaller percentage being probably due to the inclusion of slight cases, only recognized late as being diphtheria at all. Thus for eleven years no death occurred, in this author's experience, among the cases treated on the first day of the disease.

The agreement shown among these sets of figures is very striking. There is invariably a progressive increase in the mortality as the remedy is given later and later after the onset of the attack. No

stronger evidence could be found in favour of the use of antitoxin in this malady, for there is absolutely no explanation

COMPARATIVE MORTALITY IN CASES OF DIPHTHERIA ACCORDING TO THE PERIOD OF THE DISEASE AT WHICH ANTITOXIN WAS ADMINISTERED

SOURCE	FIRST DAY		SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY	
	Cases.	Deaths, cent. Mor.	Cases.	Deaths, cent. Mor.	Cases.	Deaths, cent. Mor.	Cases.	Deaths, cent. Mor.	Cases.	Deaths, cent. Mor.
Committee's Report ...	764	38 4·9	1,005	89 8·3	620	79 12·7	336	77 22·9	390	152 38·9
N. York Health Board statistics ...	126	11 8·7	215	26 12·0	228	37 16·6	153	32 20·9	203	59 29·0
Chicago Health Board statistics ...	106	0 0·0	336	5 1·5	620	18 2·7	269	38 14·1	97	33 34·0
Total ...	996	49 4·9	1,616	120 7·4	1,468	134 8·8	758	147 20·7	690	244 35·3

<sup>1</sup> *M.A.B. Rept.*, 1912, p. 192, and 1913, p. 179.

which we can adduce for this steadily increasing death-rate according to the delay in the use of the remedy, other than the hypothesis of its curative power when administered sufficiently early. This accords exactly with what is theoretically to be expected of the serum and with experimental results obtained on the lower animals.

While pronouncing thus unhesitatingly in favour of the use of antitoxin, it is necessary to bear in mind that some authorities who have had good opportunities of judging of its value are sceptical as to its usefulness. Among American writers we may mention Hermann<sup>1</sup> and Rupp,<sup>2</sup> both of whom decline to subscribe to the general verdict in favour of the remedy. On the continent of Europe Kassowitz<sup>3</sup> was equally opposed to the prevailing view. He pointed out that, although a fall in the mortality from diphtheria was experienced in many parts of the world synchronously with the introduction of antitoxin, yet latterly the death-rate had risen again in many places in spite of its continued use; hence the fall in the death-rate could not be ascribed to the antitoxin. There is much truth in this argument, as has already been admitted; and if we had only this means of judging of the value of antitoxin, it would be necessary to return a verdict of "not proven." But the evidence available as to the progressively greater mortality in cases of diphtheria, according as they are left for increasing periods of time without antitoxin, appears to constitute irrefragable proof of the value of the remedy; and the expression of doubt as to its efficacy becomes increasingly rare in medical writings.

**Mode of administration of antitoxin.**—As a rule diphtherial antitoxin is administered *subcutaneously*, but it may be injected intramuscularly (Rolleston speaks highly of this method) or intravenously (*see* pp. 51, 55).

It would seem advisable to make use of the intravenous

<sup>1</sup> *Med. Record*, Jan. 20, 1900.

<sup>2</sup> *New York Med. Journ.*, Jan. 27, 1900.

<sup>3</sup> *Therapeut. Monatsh.*, 1902, pp. 223, 499.

method of injection in cases in which the symptoms are severe, and especially in those in which the use of the serum has been unduly postponed. Good results are reported by Cairns<sup>1</sup> from this mode of procedure, very large doses of antitoxin being used in some cases (*see below*).

The antitoxin has also been given by some physicians by the *rectum* and by the *mouth*. Dominici<sup>2</sup> was one of the first to adopt these procedures, and reported five successful cases. Parkinson<sup>3</sup> states that rectal administration has been carried out at the London Temperance Hospital, and that the results obtained have been very satisfactory. He recommends this method as being free from some of the disadvantages of subcutaneous injection, such as local abscess-formation, which sometimes follow neglect of aseptic precautions. Paton,<sup>4</sup> who advises the use of diphtherial antitoxin in septic conditions not due to the *B. diphtheriæ*, administers the remedy by the mouth, stating that it is not affected by digestion; and Zahorsky<sup>5</sup> advises the administration of antitoxin in milk to children when a prophylactic dose is needed. Pilcher<sup>6</sup> also commends the oral method of administration, and Chantemesse<sup>7</sup> has given antitoxin by the rectum with satisfactory results. On the other hand, the results of experiments, from which mild infections and the possibility of coincidence have been carefully eliminated, show that this procedure (*see p. 50*) is ineffective and unscientific; and since there is no doubt that antitoxin acts well when administered by hypodermic injection, while the drawbacks to this method are very insignificant, it seems wiser to use it (or the intravenous or the intramuscular method) for treatment in all cases.

<sup>1</sup> *Lancet*, 1902, ii, 1685.

<sup>2</sup> *Gaz. degli Ospedali*, July 19, 1896.

<sup>3</sup> *Brit. Med. Journ.*, 1903, i, 1432.

<sup>4</sup> *Australas. Med. Gazette*, 1902, Feb. 20.

<sup>5</sup> *Archives of Pediatrics*, March, 1899.

<sup>6</sup> *Brit. Med. Journ.*, 1904, ii, 1751.

<sup>7</sup> *Sem. Méd.*, July, 1896.

**Prophylactic use of antitoxin.**—It has been ascertained beyond reasonable doubt that the administration of a comparatively small dose of antitoxin will produce immunity to the disease for a certain period of time. Ronald French made a series of observations upon nurses to whom 3,000 units of antitoxin—an excessive prophylactic dose—were administered before they went on duty in diphtheria wards, and found that the diphtherial opsonin-index at first rose rapidly, reached a maximum in from 4 to 11 days, then fell to a point below the normal, and returned to the normal in about 3 weeks after the injection. Netter<sup>1</sup> originally showed that the protection begins about the end of the first day (24 hours) after the injection, and lasts for about 3 weeks. Beyer<sup>2</sup> states that after 24 hours the amount of antitoxin present in the blood is only one-half to five-sixths of the amount injected; after 2 to 4 days about one-quarter to one-half the quantity remains; after 5 or 6 days there is still one-third to one-quarter; and none is present at the end of 3 weeks. The protective substance is possibly excreted in the urine, etc., or possibly anti-antitoxins or destructive ferments may be formed. There seems no reason to hold that the length of the immunity is at all proportional to the dose administered.

In the presence of an epidemic of diphtheria, among 491 children exposed to infection, who did not receive protective injections, 87 contracted the disease; while of 502 children, who had been similarly exposed and were given protective injections, only 13 became infected. Of these, 7 developed the disease within 24 hours, and 6 more than a month after the injections. Since the immunity does not begin for 24 hours and passes off in approximately 3 weeks, these figures strongly support the prophylactic use of the remedy.<sup>3</sup>

As a result of Netter's communication and the discussion

<sup>1</sup> *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902

<sup>2</sup> *Deut. med. Woch.*, 1912, No. 50.

<sup>3</sup> Netter, *loc. cit.*

which followed it, the Academy of Medicine (Paris) passed resolutions<sup>1</sup> to the following effect:—

1. Preventive injections of 1,000, or at most 2,000, units of diphtherial antitoxin produce immunity to the disease. This protection is transitory in character.
2. Such preventive injections are especially to be recommended in the members of families in which cases of diphtheria have occurred, in order to immunize the other children.
3. They are also called for in schools, crèches, hospitals, etc., where children are collected together so that infection is easily spread from one to another.
4. Injections of antitoxin are useful in patients suffering from scarlet fever and measles, in which affections diphtheria is a frequent complication.
5. The prophylactic use of antitoxin does not preclude the carrying out of ordinary measures of disinfection and isolation.

American writers are strongly in favour of the preventive use of antitoxin. Biggs<sup>2</sup> states that out of 3,109 cases in which this was given, only 9 children acquired diphtheria; and Park<sup>3</sup> records 6,506 cases of immunization, among which 28 developed the disease within 24 hours, before the protection was effective, while only 27 were attacked after this limit of time, of whom one died of scarlet fever. Billings<sup>4</sup> attributed a rise which had occurred in the diphtheria-rate to the neglect of the prophylactic use of the remedy.

On the other hand, Violi<sup>5</sup> considers that antitoxin is not a sure preventive of diphtheria, and advises that it

<sup>1</sup> *Brit. Med. Journ.*, 1902, i. 997.

<sup>2</sup> Quoted by Billings, *op. cit.*

<sup>3</sup> *Journ. of the Amer. Med. Assoc.*, 1900, i. 902.

<sup>4</sup> *New York Med. Journ.*, 1900, lxxi. 234.

<sup>5</sup> *Pediatrics*, June, 1900.



should only be given when there is a certainty that a child has been exposed to infection. The difficulty which exists in judging of the value of any prophylactic has been emphasized by Peters,<sup>1</sup> who records instances of failure to protect persons in whose fauces the *B. diphtherie* had actually effected a lodgment. Netter<sup>2</sup> admits that prophylactic injections need not be given if the children can be kept under observation and apart from others whom they might infect.

It might be well, owing to the transient nature of the immunity obtained, to repeat the dose if the epidemic continued, so as to renew the protection. Even apart from re-infection, virulent bacilli have been known to remain present in the throats of children for long periods of time.

It cannot be denied that there is a real, though exceedingly small, risk in the administration of antitoxin. Nevertheless, as 500 units is an efficient prophylactic dose, and can be obtained in half a cubic centimetre of serum, even in private practice among well-to-do patients it seems advisable to give preventive injections as a routine procedure. In the case of institutions, such as schools and hospitals, the wisdom of this course is even clearer; here there is a very great liability for the infection to spread from one patient to another, and this precautionary measure seems imperatively necessary. It is well to remember the frequency with which epidemics of diphtheria are kept alive by means of children who are the subjects of chronic nasal discharges. This condition seems to have little effect on the health of the child itself, but the virulent bacilli contained in the nasal secretion are capable of infecting others. Hence, children who have been brought into contact with such "carriers" must be looked upon as having been exposed to infection and treated accordingly. In the management of epidemics of diphtheria, particularly those which may

<sup>1</sup> *Brit. Med. Journ.*, 1907, ii. 865.

<sup>2</sup> *Loc. cit.*

be designated "school epidemics," the most expeditious, economical, and efficient method is undoubtedly that which includes the examination of swabbings from the entire community, segregation of all carriers, and the prophylactic injection of antitoxin into all contacts.

**Active prophylactic immunization.**—Behring<sup>1</sup> has introduced a method of active immunization against diphtheria, to take the place of prophylactic injections of antitoxin. He makes mixtures of toxin and antitoxin of various strengths, called respectively MM<sub>1</sub>, M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>, the exact composition of which we have not been able to find stated. The injections are given subcutaneously or intradermally between the shoulders. Local redness and infiltrations, with swelling of lymphatic glands, fever, and headache, are produced (Kleinschmidt and Viereck).<sup>2</sup> Kissling,<sup>3</sup> who gave doses of 0.1 to 0.3 c.c. of a 1-in-5 solution of MM<sub>1</sub>, states that there is no negative phase, but that it takes some days for immunity to develop. He quotes statistics of 310 cases. Of these, 111 patients received two injections, and none of them developed diphtheria, while 199 received only one injection, and among these there were 8 cases of infection, but 3 of these occurred within the first nine days after treatment, before immunity could be supposed to be complete. There is not yet available sufficient evidence on which to judge of the merits of Behring's method. It is difficult to believe the statement that there is no period of increased susceptibility (negative phase) at the beginning of the treatment.

**Dose of antitoxin.**—The tendency at the present time seems to be in the direction of giving large doses of antitoxin. For **prophylactic** use 150 units were at first generally employed in America, but this dose was found to be too small, and the Health authorities<sup>4</sup> there now

<sup>1</sup> *Congr. f. inn. Med.*, Wiesbaden, 1913.

<sup>2</sup> *Deut. med. Woch.*, 1913, p. 1977.

<sup>3</sup> *Ibid.*, p. 2500.

<sup>4</sup> Billings, *New York Med. Journ.*, 1900, lxxi. 234.

recommend the use of not less than 300 units. Jump<sup>1</sup> recommends 250 units for children under 2 years, 500 for older children and adults. The Paris Academy, whose resolutions were alluded to on p. 119, speak of 1,000 to 2,000 units as a protective dose. There can be little doubt that these last are unnecessarily large. Perhaps, on the ground of American experience, we may consider that 300 to 500 units is the proper dose—no distinction being made between children and adults; or if a distinction is made it should be in the direction of an increased dose for the infant. It seems advisable to repeat the dose, if liability to infection continues, or if virulent bacilli should still be found in the child's throat.

For purposes of **treatment** much larger amounts are required. Villy<sup>2</sup> advises that in cases of moderate severity 2,000 units should be given at once and repeated in 12 hours, if necessary. In severe cases, 8,000 to 12,000 units may be the first dose, followed by 2,000 to 8,000 units every 12 hours. McCollom<sup>3</sup> gave an initial dose of 8,000 units in a severe and apparently hopeless case, following this up with 4,000 units some hours later, and repeating the dose every 4 to 6 hours, till 92,000 units in all had been administered. The patient recovered completely. Satterthwaite<sup>4</sup> states that the initial dose for an infant under 1 year is now established at 2,000 units; for one over 1 year, 3,000; and for an adult, 4,000 to 6,000 units. Cairns<sup>5</sup> puts the doses for subcutaneous injection at from 4,000 to 20,000 units; while intravenously he administers from 20,000 to 35,000 units. He holds that in severe cases an initial dose of 20,000 units is not excessive. Welden<sup>6</sup>

<sup>1</sup> *Philad. Med. Journ.*, Jan. 11, 1902.

<sup>2</sup> *Med. Chron.*, 1900, ii. 241.

<sup>3</sup> Quoted by Satterthwaite.

<sup>4</sup> *Med. News*, May 16, 1903, p. 936.

<sup>5</sup> *Lancet*, 1902, ii. 1685.

<sup>6</sup> *New York Med. Journ. and Philadelphia Med. Journ.*, Nov. 14, 1903, p. 927.

finds that the best results are obtained by administering 2,000 units every three hours until the severity of the symptoms diminishes.

DOSE OF DIPHTHERIAL ANTITOXIC SERUM (IN UNITS)  
RECOMMENDED BY VARIOUS WRITERS

Author	Mild case	Average case	Severe case	Prophylaxis
Cohn <sup>1</sup> . .	1,000	—	1,500-2,000	—
Baginsky <sup>2</sup> . .	1,500	4,000	5,000	—
Bishop <sup>3</sup> . .	{ 2,000 (adult) 4,000 (child)	—	12,000 (max.)	—
Rudolph <sup>4</sup> . .	—	3,000 repeatd.	—	300-500
Berg <sup>5</sup> . .	—	3,000 repeatd.	—	—
Pexa <sup>6</sup> . .	—	3,000	—	—
Fischer <sup>7</sup> . .	2,000	5,000	10,000	—
Souza <sup>8</sup> . .	—	7,000	—	—
McCullom <sup>9</sup> . .	4,000	8,000 repeatd.	12,000 repd.	—
Berlin <sup>10</sup> . .	—	4,000-8,000	16,000	—
Rolleston <sup>11</sup> . .	{ 3,000-12,000	12,000-18,000	18,000-24,000 (repeated)	—
Park <sup>12</sup> . .	—	6,000-10,000	20,000-30,000	—
Ibrahim <sup>13</sup> . .	—	—	—	250-500
Wesener <sup>14</sup> . .	—	—	—	300-400
Aaser <sup>15</sup> . .	—	—	—	300-400

On the other hand, some authorities recommend the use of small doses for treatment. Thus Musser <sup>16</sup> advises that children from 6 to 8 years old should receive only 500 units,

<sup>1</sup> *Mitt. u. d. Grenzgeb. d. Med. u. Chir.*, Bd. xiii., Hft. 4 and 5.

<sup>2</sup> *Berlin. klin. Woch.*, 1908, p. 1257.

<sup>3</sup> *Brit. Med. Journ.*, 1907, ii. 1528.

<sup>4</sup> *Ibid.*, 1903, i. 1078. <sup>5</sup> *Med. Record*, Nov. 26, 1904.

<sup>6</sup> *Abstr. in Centralbl. f. inn. Med.*, 1905, p. 975.

<sup>7</sup> *Med. Record*, 1904, lxvi. 875.

<sup>8</sup> *Gaz. dos Hospitales do Porto*, Aug., 1907.

<sup>9</sup> *Med. Record*, loc. cit. <sup>10</sup> *Deut. med. Woch.*, 1910, No. 5.

<sup>11</sup> *Practitioner*, 1904, lxxiii. 615 *et seq.*

<sup>12</sup> *Boston Med. and Surg. Journ.*, Jan. 16, 1913.

<sup>13</sup> *Deut. med. Woch.*, Mar. 16, 1905.

<sup>14</sup> *Münch. med. Woch.*, 1905, No. 12.

<sup>15</sup> *Berlin. klin. Woch.*, Sept., 1905.

<sup>16</sup> *University Med. Magazine (Philadelphia)*, March, 1900.

while those over 8 should have 1,000, repeated if necessary in 8 to 12 hours. Geffrier and Rozet<sup>1</sup> also recommend that small doses should be used; and these writers do not advise the use of prophylactic injections, on account of their occasional bad effects.

Reiche<sup>2</sup> states that when large numbers of cases are taken for statistical purposes no advantage appears to be derived from the use of large doses.

Our own opinion inclines to the administration of an initial subcutaneous dose of 2,000 units for an infant, and 4,000 for an adult, repeated 12 and 24 hours later, if necessary; this may be accompanied, if the case is a very severe one or is first seen at or about the fifth day, by an intravenous injection of a similar amount.

The opinion has been expressed that the use of antitoxin after the fifth day of the disease is ineffectual and should be discontinued.<sup>3</sup> Its use in late stages has even been looked upon as actively harmful. Such views are vigorously combated by Rolleston,<sup>4</sup> and are contrary to the accepted theory of the nature of the disease. We believe that in any severe case of diphtheria antitoxin should be administered as soon as the case comes under treatment. In cases of relapse serum should again be injected in spite of the probability that severe symptoms of "serum-disease" may ensue. It is well, however, to bear in mind the condition named by Rolleston "angina redux" (the *angine de retour* of French writers), a form of simple tonsillitis which does not call for specific treatment (*see* p. 128).

**Antitoxin and post-diphtheritic paralysis.**—There is reason to believe that since the introduction of antitoxin the percentage of cases which suffer from paralysis after diphtheria has definitely increased. This effect was at first attributed to the remedy, and some prejudice

<sup>1</sup> *Arch. de Méd. des Enfants*, Feb., 1900.

<sup>2</sup> *Med. Klinik*, 1913, pp. 11, 57.

<sup>3</sup> Bolton, *Lancet*, 1907, i. 870.

<sup>4</sup> *Ibid.*, 1907, i. 1112.

against the use of it was thereby excited. Since, however, as we have just endeavoured to prove, it saves the lives of many patients who would otherwise have died, and these severe cases are those which are most likely to exhibit paralysis later on, there inevitably arises an increase in the percentage of cases of paralysis ; it is in reality a testimony to the value of the antitoxin, and not a drawback to its use.

With regard to the incidence of paralysis after diphtherial intoxication, Ransom<sup>1</sup> comes to the following conclusions as the result of experimental researches :—

“ 1. Paralysis may certainly be expected after intoxication with not less than one-quarter of a minimal fatal dose. With doses between one-quarter and one-eighth of this amount paralyzes occur but are not constant, and below one-eighth no paralysis was noticed.

“ 2. The larger the dose of toxin the severer will be the paralysis, if the animal survives long enough.

“ 3. Neutralized mixtures of toxin and antitoxin containing only about one lethal dose or less do not appear to cause paralysis.

“ 4. Antitoxin, given 15 to 22 hours after intoxication with doses of toxin not greater than the lethal dose, exercises in large doses a mollifying influence on the subsequent paralysis. . . . Small doses of antitoxin have no evident effect in diminishing the paralysis.

“ 5. Transferring the results to practice among human beings, we may expect liberal doses of antitoxin given early in the illness to influence favourably the subsequent paralysis ; and this favourable influence is likely to manifest itself, not so much in the local paralyzes (soft palate, etc.) as in such fatal symptoms as failure of the heart. Severe cases are, however, likely to be followed by some paralysis in spite of even large doses of antitoxin.”

It would appear, then, that so far from having any part in the production of paralysis, antitoxin has some power of restraining it. These results are in accordance with those

<sup>1</sup> *Journ. of Pathology and Bacteriology*, 1900, vi. 397.

of other writers.<sup>1</sup> Thus Rosenau and Anderson found that in guineapigs one unit of antitoxin given before or at the time of infection entirely prevented paralysis; that if it were given within 24 hours it modified the subsequent paralysis; but that at later periods it had no influence. Comby,<sup>2</sup> on the other hand, advises the use of antitoxin in the treatment of paralysis which has actually occurred.

PERCENTAGE INCIDENCE OF PARALYSIS ACCORDING TO DAY OF  
TREATMENT WITH ANTITOXIN <sup>3</sup>

Approximate date of treatment	Petit	Monti	Reichsfeld	Rolleston
1st day	—	—	—	5.5
2nd "	6.25	—	25	15.09
3rd "	19	8	33	18.07
4th "	24.70	12	—	28.07
5th "	—	33.3	50	35
6th "	—	50	—	34
7th "	38.70	66.2	—	19

**Ill effects of antitoxin.**—The manner in which the injection of the serum of any species of animal into an individual of another species is liable to be followed by toxic symptoms has already been considered (p. 55).

It cannot be denied that in a certain number of instances the injection of diphtherial antitoxin has been followed by *death*, directly attributable to the action of the serum. A melancholy instance was afforded by the sudden death of Professor Langerhans' infant son soon after a prophylactic dose of the serum, but this has been attributed

<sup>1</sup> Malynicz ("Ueber d. Häufigkeit der postdiphtherischen Lähmungen vor u. nach Serum-behandlung," Zurich, 1908) gives statistics to show that not only is paralysis more common after serum treatment, but that cardiac paralysis is especially common and fatal. We do not think that this is the usual experience.

<sup>2</sup> *Lancet*, 1906, i. 54, 243.

<sup>3</sup> Rosenau and Anderson, Pub. Health and Mar. Hosp. Service, U.S.A. *Bull. Hyg. Lab.*, No. 38, 1907.



to the existence in the child of the status lymphaticus, which predisposes to death from trivial causes. A similar case of sudden death is recorded by Boone<sup>1</sup> in a boy aged 10 years, who after an injection of 4,000 units of antitoxin suddenly sat up, clutched at his throat, became deeply cyanosed, and died. No cause of death was discovered at the necropsy. Mackeen<sup>2</sup> reports a fatality in a girl aged 17, the subject of asthma and status lymphaticus: in this case the antitoxin was administered as a prophylactic. Most of the fatal cases recorded have been of the same sudden character. A case in which death took place at a later period, and was due to the same vascular disturbance which gives rise to the rashes often seen after injections of antitoxin, is recorded by Gerlach.<sup>3</sup> In this instance an erythematous eruption appeared on the eleventh day after the injection; on the twelfth day there were clonic spasms, without the presence of albumin in the urine or any symptoms of uræmia. At the necropsy there was found an extradural meningeal hæmorrhage, which was attributed to a leakage from the vessels, analogous to the escape of serum, and sometimes of blood, seen in erythema multiforme. Gerlach alludes to another case in which *cerebral symptoms* came on after an injection, but which did not end fatally.

Holladay records a case<sup>4</sup> in which a man of 26 received an injection of only 500 units. This was followed by tingling in the arm, where the dose had been administered, cyanosis, constriction at the chest, and collapse; recovery subsequently ensued.

Saward<sup>5</sup> records 2 cases in which sudden *syncope* occurred after injections of antitoxin. It must be remembered, however, that the toxin of diphtheria acts on

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1908, l. 453.

<sup>2</sup> *Boston Med. and Surg. Journ.*, April 6, 1911.

<sup>3</sup> *Therapeut. Monatsh.*, April, 1903, p. 198.

<sup>4</sup> *Virginia Med. Semi-Monthly* (quoted in *Indian Lancet*, March 23, 1903, p. 481).

<sup>5</sup> *Brit. Med. Journ.*, 1902, i. 1025.

the cardiac muscle, producing a tendency to syncopal attacks; it is therefore very doubtful whether these cases were really to be attributed to the antitoxin, since they might equally well have been caused by the disease itself.

An **exudative tonsillitis** may occur as a serum-phenomenon (*angina redux*, Rolleston), and may give rise to fears of a relapse; there are, however, no diphtheria bacilli in the exudate, and this is not a distinct membrane but a soft deposit. It is important to distinguish this condition, which only occurs with other manifestations of serum disease, from a true relapse, as it would obviously be improper to inject more antitoxin.

**Swelling of the cervical glands** is another rare effect of serum treatment. Bolton<sup>1</sup> alludes to the occurrence of *abscesses* at the site of injection, and in one case *necrosis* of the overlying skin. Such incidents are, however, probably due to some failure of antiseptic precautions.

The most frequent ill effects brought about by injection of serum are **cutaneous eruptions**<sup>2</sup> (*see also* p. 57). Park<sup>3</sup> observed them in 3 per cent. of his cases, Stanley<sup>4</sup> in over 25 per cent., and Villy<sup>5</sup> in 35·2 per cent. The rashes are generally classified as (1) erythematous, (2) scarlatiniform, (3) morbilliform, and (4) urticarial. They are all manifestations of a condition which may be termed erythema multiforme, consisting in a tendency to vascular dilatation of different degrees and distribution, and escape of serum into the tissues (urticaria). It appears that different brands of serum tend to produce different types of eruption.

1. *Erythema*, or simple localized hyperæmia, is generally

<sup>1</sup> *Lancet*, 1899, i. 891.

<sup>2</sup> Galitsis, Thèse de Paris, quoted in *Journ. de Méd. et Chir. Pratiques*, Sept. 25, 1903, p. 692, states that some eruptions occurring in diphtheria are due to infection with a special organism, *Diplococcus hemiphilus*, and are wrongly attributed to the serum.

<sup>3</sup> *Brit. Med. Journ.*, 1902, i. 386.

<sup>4</sup> *Op. cit.*

<sup>5</sup> *Op. cit.*

regarded as the commonest form of rash. It may take the shape of a slight blush, either at the point of injection or elsewhere, or may consist of slightly raised circinate patches, which may coalesce to form gyrate patterns. Favourite seats of this variety are the extensor surface of the limbs. Out of 112 cases of rash noted by Stanley, 58 were simple erythema. Its average date of appearance after the injection was the twelfth day, varying from the fourth to the twenty-ninth. It may be combined with urticaria (15 cases).

2. The *scarlatiniform* rash is a more pronounced form of the erythematous; it is more intense and becomes widely generalized. Stanley noted this variety in 6 cases out of 112. It is often followed by desquamation. Leiner<sup>1</sup> states that this form of eruption tends to come out within the first five days after injection—an earlier period than that of most of the other varieties; that it starts from the point of injection; that it is followed by peeling; that it is contagious, and that it seems to protect against infection with scarlatina. The infection is difficult to eradicate from a ward in which cases have occurred. He concludes that it is true scarlatina, and compares it with the surgical variety of this disease. It is very possible that the cases observed by Leiner were of this nature, and there is no doubt that the diagnosis between a serum rash and an attack of true scarlet fever must be difficult; but there is no reason to suppose that all cases of scarlatiniform rash after the administration of antitoxin are of this character. The coexistence of diphtheria and scarlatina is, however, not very infrequent.

3. *Morbilliform eruptions* formed less than 3 per cent. of Stanley's cases. They may be accompanied by swelling of the face, conjunctivitis, and lachrymation, so as exactly to resemble measles. Even pyrexia may occur. Distinguishing points from true measles are that the rash comes out first on the limbs, instead of on the face and

<sup>1</sup> *Wien. klin. Woch.*, 1902, No. 43.

behind the ears; that gyrate patterns are met with; and that there is no accompanying bronchitis (Villy).

4. *Urticarial eruptions* are common. They occurred in 30 of Stanley's 112 cases. They are met with at an earlier period after injection than the erythemata—from the fourth to the nineteenth day (average, ninth, Stanley). The urticaria may be quite transient, or may last several days. The itching is sometimes very severe. A local urticaria round the site of injection may be seen.

*Pains in the joints*, often accompanied by swelling, erythema, and pyrexia, are another inconvenience which may arise after injections of serum. They are not usually severe, but Taillens<sup>1</sup> records two cases in which they were accompanied by high fever and a rash; in one of his cases the pains were so intense that the child could not move at all, or bear the weight of the bed-clothes to rest on her. Villy noted joint-pains in 6·5 per cent. of his cases.

Successive outbreaks of cutaneous eruptions or attacks of joint-pains often correspond with successive administrations of serum.

*Albuminuria* may follow the use of antitoxin, but is not of any serious import. Actual *nephritis* is said to be rather diminished than increased by the use of this remedy, and certainly existing albuminuria does not contraindicate the use of antitoxin. *Suppression of urine* is also more rarely seen in these days (Villy). *A rise of temperature* as a result of the antitoxin was observed by this writer in 19·8 per cent. of his cases.

An interesting case illustrating the effects of *idiosyncrasy* in relation to antitoxic serum is reported by Reckles.<sup>2</sup> He administered an injection of 4,000 units of antitoxin to a woman who was suffering from faucial diphtheria. A few days later, when she appeared to be convalescent, a severe urticaria developed, and shortly afterwards she was seized

<sup>1</sup> *Revue Méd. de la Suisse Romande*, July 20, 1903, p. 463.

<sup>2</sup> *Quarterly Med. Journ.*, Feb., 1903.

with a sudden attack of præcordial pain and dyspnœa, with marked lividity of the face. In the next few days nine successive attacks of this nature were experienced, but in the end the patient recovered satisfactorily. It was subsequently learnt that two years previously she had received an injection of antitoxin for diphtheria, and that she had on that occasion also suffered from attacks of dyspnœa and cyanosis; and the question naturally arises as to whether these symptoms, though late in appearing, can be attributed to anaphylaxis.

Borchman<sup>1</sup> states that antitoxic serum produces less ill effects if it is warmed to 58° C. before it is injected. This has only a slight effect in diminishing its potency. He quotes his experience in 578 cases; in 193 of these the remedy was given in the ordinary way, cold, and among these there were 22·7 per cent. of rashes. On the other hand, among 385 patients who received the warm serum, only 16·3 per cent. developed these troubles. Courmont<sup>2</sup> reports that no rashes or anaphylaxis follow the intravenous injection of serum. Rolleston regards the occurrence of serum-rashes as of good prognostic import.

**Antitoxin in conjunctival diphtheria.** — Antitoxin has proved of the greatest service in diphtherial infection of the conjunctiva. Emmett Holt<sup>3</sup> states that without it total destruction of the eye generally results, whereas with its aid good results may be obtained. He administers 2,400 units as a dose. Stevenson<sup>4</sup> also bears testimony to the value of the remedy, as the result of a study of 43 cases; he advises the local use, along with the antitoxin, of a lotion of perchloride of mercury (1 : 5,000). The diphtherial nature of the affection must be established

<sup>1</sup> *Dietskaya Meditsina*, 1900, v., No. 3. (Abstr. in *Pediatrics*, 1900, vol. x., p. 316.)

<sup>2</sup> *Soc. Méd. des Hôp.*, 1905, p. 504.

<sup>3</sup> *Pediatrics*, May, 1902.

<sup>4</sup> *Brit. Med. Journ.*, 1902, i. 720. Cf. Strzemiński, *Recueil d'Ophthalmol.*, Oct., 1905.

by bacteriological examination, including inoculation experiments, the morphological resemblance between *B. diphtherie* and *B. xerosis* being a fruitful source of error.

**Post-scarlatinal ear-disease.**—On the other hand, curiously enough, in some cases of middle-ear disease following scarlatina, in which diphtheria bacilli appear to be the pathogenic organisms, antitoxin is said to act neither as a prophylactic nor as a curative agent, although the patients seem to obtain immunity to faucial infection by the bacilli.<sup>1</sup>

**Cancrum oris.**—Some cases of noma, or cancrum oris, are due to the *B. diphtherie* and should be treated with antitoxin. Telford<sup>2</sup> records a successful case.

**Diphtheria as a complication of other diseases.**—Diphtheria is liable to occur as a complication of scarlet fever and of measles. In the former malady the supervention of diphtheria constitutes a very grave condition, the mortality being very high. In measles, also, infection with diphtheria is very likely to occur. It has therefore been suggested that prophylactic injections of antitoxin should be given as routine treatment in both these diseases. If many cases of such superinfection have occurred in a scarlet-fever ward, then such treatment would certainly be advisable. Heubner<sup>3</sup> states that a larger dose of the serum is needed to produce immunity in cases of measles than under other conditions (twice the amount), and that the duration of the protection is also shorter. Richardière<sup>4</sup> records that in the first four months of the year 1901 two to four cases of diphtheria used to occur each month in the measles-ward of a hospital. After this time all the children as they came into the ward were injected with antitoxin, and no more cases of diphtheria occurred.

<sup>1</sup> Duncan Forbes, *Journ. of Pathol. and Bacter.*, 1903, viii. 448.

<sup>2</sup> *Med. Chron.*, 1906, p. 221.

<sup>3</sup> Quoted by Netter, *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902.

<sup>4</sup> *Ibid.*

**Nasal diphtheria.**<sup>1</sup>—The ordinary acute faucial diphtheria may spread to the nose, and such cases show a very high rate of mortality. Large doses of antitoxin are called for by way of treatment. There is also a chronic nasal inflammation associated with the formation of membrane (membranous rhinitis), which affects the nose, and in which virulent diphtheria bacilli are found. The patients do not suffer from the toxic symptoms characteristic of diphtheria, nor do they exhibit, as a rule, any distinct signs of ill health; but the discharge from the nose is capable of conveying the infection to others. Indeed, it is probable that many epidemics of diphtheria, the origin of which has been difficult to understand, might be traced to such chronic nasal disease, that may pass as an ordinary "cold in the head." Antitoxin often acts successfully in these cases; at other times antibacterial serum (p. 134) is useful.

**Diphtherial antitoxin in other diseases.**—Various writers have detailed their experience with antitoxin in diseases other than diphtheria. Thus Talamon and Gay have used it in pneumonia; Paton recommends it for all septic conditions, and thinks it has a definite influence on inflammatory tissue; Schapiro and Tsvietaieff, and Mastri and Tomaselli have tried it in erysipelas; and Konarzsherski believes that it will cure whooping-cough. Del Monaco believes it to be efficacious in aphthous stomatitis, Sizemski in ozaena, and Gillett used it with benefit in a case of asthma—not a microbial disease. It is difficult to take

<sup>1</sup> In this connection we may note that Mygind (*Journal of Laryngology*, Aug., 1898) made use of injections of diphtherial antitoxin in 10 cases of ozaena. He found that the injections produced congestion of the mucosa of the nose and subsequent formation of crusts, while the fetid odour disappeared. It does not seem to be suggested that the *B. diphtheriae* is the cause of ozaena, the nature of which is not well understood. Mygind's experience with antitoxin is analogous to that of other observers in the different maladies alluded to in the text, in which it is difficult to believe that the good results seen were in reality due to the antitoxin. Our present knowledge of the action of serums is, however, too small to enable us to pronounce with any degree of confidence as to what they can or cannot do.



these results seriously, since the most definitely established fact at present with regard to serums is their specific nature, i.e. the power possessed by each of counteracting the poisons or killing the bacteria of that disease alone for which it is manufactured. It has, indeed, been suggested that diphtherial antitoxin may act as a stimulant to the production of leucocytes generally, and so may be useful in other diseases in which leucocytosis is beneficial, much in the same way as cinnamic acid is said to act in tuberculosis. Thus R. French has observed a gradual but steady rise in the tuberculo-opsonic index following the administration of 3,000 units in a case of tuberculous disease of the hip. Bradshaw<sup>1</sup> records a distinct rise in the tuberculo-opsonic index shortly after an injection of anti-diphtherial serum, followed, however, by a marked fall, from one to three months later. In spite of these observations, the probabilities point at present rather to the supposed benefits of antitoxin in other diseases being due to fallacies of observation, which are difficult to avoid in studying the action of any remedy.

#### ANTIBACTERIAL SERUM

Antitoxic serum, as already explained, does not tend to kill the bacilli which cause diphtheria. They will grow readily in the fluid itself, and continue to exist in a virulent form in the throats of persons who have been injected with antitoxin. Wassermann<sup>2</sup> claims to have succeeded in the production of a serum which is bactericidal. He prepares a fluid somewhat analogous to Koch's "new tuberculin," by pounding up the bacilli and extracting them with ethylene-diamine, 20 c.c. of this solvent being added to 1 grm. of pulverized bacilli. The mixture is well shaken in a special apparatus, and is then submitted to the centrifuge. The supernatant fluid is of a yellow colour, and contains the intracellular toxins of

<sup>1</sup> *Lancet*, 1906, i. 1387.

<sup>2</sup> *Deut. med. Woch.*, Oct. 30, 1902.

the bacilli. It is capable of killing rabbits when injected into them. If, however, the toxin is mixed with a proportion of antitoxin and repeatedly injected into these animals, the serum obtained from them is strongly agglutinative of diphtheria bacilli. Wassermann thinks that this serum will afford a means of distinguishing *B. diphtheriae* from pseudo-diphtheria bacilli, and that it may possibly have curative properties.

Concetti<sup>1</sup> writes in favour of the use of antibacterial serum as an adjunct to antitoxin, and as a local application to the throat in the form of lozenges. A "bivalent" (antitoxic and antibacterial) serum may also be used prophylactically, as well as for irrigation of the nostrils to avoid spread of infection to the nose; for local application to the trachea after tracheotomy (2.5 c.c. every three hours); and in conjunctival diphtheria (Bandi).<sup>2</sup> Dopter<sup>3</sup> advises the local use of antitoxic serum in the form of lozenges; but this serum is theoretically inferior to the bivalent serum. He also uses insufflations of powdered dry serum for nasal diphtheria, and finds this procedure of some value, but less so than in faucial cases.

#### VACCINE TREATMENT

Possessing, as we do, in antitoxic serum a remedy at once so potent and so reliable, and one, moreover, which has the overwhelming advantage of being available at a moment's notice, it is not surprising to find that diphtheria vaccine has not been employed therapeutically to any large extent. At the same time vaccine treatment is of distinct value in those infections which become chronic, and in which the responsible organism remains domiciled in the throat or nose for prolonged periods after the acute clinical symptoms have been entirely removed by the employment of antitoxin ("carriers"). In such cases a vaccine should be prepared

<sup>1</sup> *Riv. di clin. Pediatr.*, 1905, No. 6.

<sup>2</sup> *Il Policlin.*, July, 1906.

<sup>3</sup> *Gaz. des Hôp.*, 1905, p. 459.

from that strain of *B. diphtherie* actually infecting the patient, the bodies of the bacilli carefully washed free from toxin, the vaccine then standardized, and administered in doses of 5 to 10 millions at intervals of three to five or seven days. In our experience one to two injections are usually sufficient to ensure the disappearance of the bacilli from the local site of infection. Hewlett<sup>1</sup> uses for the same purpose endotoxin obtained from the diphtheria bacillus by grinding at the temperature of liquid air in doses of 0.5 mg.

### DIAGNOSIS

Bacteriological examination of the membranous exudation with the demonstration of the presence of the diphtheria bacillus is the only reliable method of diagnosis, although many other methods have from time to time been advocated. Schich<sup>2</sup> has suggested the subcutaneous injection of small doses of diphtheria toxin as a test for the existence of the infection, stating that susceptible individuals exhibit redness at the seat of inoculation, which appears in from twenty-four to forty-eight hours, lasts from seven to ten days, and as it fades is followed by scaling of the skin and a persistent brownish pigmentation.

**Agglutination.**—The agglutination of diphtheria bacilli is not very easily obtained, as the bacteria naturally occur in masses, closely adherent together. Lubowski<sup>3</sup> obtained the bacilli in a state of division by shaking up an emulsion of them with small glass balls, and diluting the resulting fluid with a 10 per cent. solution of glycerin. The reaction is of no practical value in the diagnosis of diphtheria.

### CONCLUSIONS

1. *Prophylaxis.*—Diphtherial antitoxin has a definite power of preventing the onset of diphtheria. The prophylactic dose should be 500 units. Antitoxin should be used

<sup>1</sup> *Lancet*, 1915, i, 275.

<sup>2</sup> *Boston Med. and Surg. Journ.*, 1914.

<sup>3</sup> *Zeitschr. f. Hygiene*, 1900, Bd. xxxv.

with this object in institutions where children are congregated together, if there is any tendency to an endemic prevalence of the disease in the institution. In other cases, if the children can be kept under close observation these protective doses are not absolutely necessary, as the prompt administration of the serum on the first appearance of the disease is an almost absolute safeguard against a fatal issue. If "swabs" can be taken from the throats of children exposed to infection, and examined for bacilli, the prophylactic doses of serum need only be given to such as exhibit the organisms in their fauces.

2. *Treatment.*—The curative effects of the serum are well established. The remedy should be given as early as possible in the course of the disease, as the mortality is progressively greater according as the serum is administered on later and later days. Therefore, if the clinical appearances are those of diphtheria, it is advisable to secure material for bacteriological examination, but to *administer antitoxin without waiting for the bacteriological report.* The initial dose for an ordinary case need not exceed 4,000 units. In severer cases much larger doses may be given. The dose may be repeated as often as necessary at intervals of four, six, twelve, or twenty-four hours. Serum should be re-administered in case of relapse, but true relapse must be carefully distinguished from simple angina redux.

3. *Method of administration.*—In ordinary cases subcutaneous or intramuscular injection of the serum is advisable. In very severe cases it may be given intravenously (warmed), in order to get the patient under its influence as soon as possible. The efficacy of the use of antitoxin by the mouth or rectum needs further elucidation. In the present state of our knowledge it is hardly justifiable to make use of this method of administration.

4. Ordinary measures of prophylaxis (isolation, etc.) and of treatment should not be omitted because antitoxin is used.

## CHAPTER VI

### TETANUS

**Causal organism.**—The micro-organism (*B. tetani*) which is the cause of tetanus was discovered by Nicolaier in 1884, and was first isolated in pure culture by Kitasato in 1890. The bacillus, in the form of spores, is widely distributed in nature, being commonly found in cultivated (manured) soils. Joseph<sup>1</sup> and Lukas<sup>2</sup> record its frequent presence in the intestinal contents of horses and cattle.

Entering the body by a wound in the skin or mucous membrane,<sup>3</sup> the bacilli do not become generalized by means of the blood-stream,<sup>4</sup> but remain localized at the point of infection, where they form their toxins. These are taken up by the peripheral nerves, and are carried along the axis-cylinders to the central nervous system. Exactly the same mode of conveyance seems to hold good in the case

<sup>1</sup> *Zeitschr. f. Infektr. u. Hyg. der Haustier*, 1910, vii. 96.

<sup>2</sup> *Zeitschr. f. Tiermed.*, 1914, xviii. 17.

<sup>3</sup> Commercial gelatin is very liable to contain the spores of tetanus, and a number of cases have been recorded in which the disease occurred after gelatin injections made for the treatment of aneurysm. An outbreak also occurred in the United States, due to contamination of diphtherial antitoxin with the toxins of tetanus. Cases have occurred after vaccination, either due to the presence of the organisms or their spores in vaccine-lymph, or to subsequent inoculation of the vaccination lesions from accidental sources. Vaccination wounds may perhaps afford a specially favourable nidus for the organisms, as they form an ulcerated surface beneath a scab. Undeserved discredit was thrown upon Haffkine's method of antiplague vaccination by some cases of tetanus arising from neglect of antiseptic precautions in the use of his prophylactic.

<sup>4</sup> Lambert (*Med. News*, 1900, July, p. 12) states that the bacilli may wander into the general circulation.

of the poison of hydrophobia, and it seems that there must be a continual streaming of the protoplasm of the nerve-fibrils towards the cells from which they are prolongations. Resection of a portion of a nerve may prevent the onset of tetanus in animals, if only a very small dose of the toxins has been administered. Part of the poison is absorbed into the lymph and blood, and, reaching the general circulation, may pass directly to the ganglion cells of the cord, or indirectly by means of peripheral nerves.<sup>1</sup> Meyer and Ransom,<sup>2</sup> as the result of many ingenious experiments, consider that it is only by the peripheral nerves that the poison can reach the spinal cord or brain. Poison circulating in the blood is taken up by the nerve-endings, and so passed on to the central portions of the nervous system, but it does not penetrate directly into the latter from the lymph. They thus explain the greater part of the incubation period met with in poisoning by tetanus toxins. They point out that the incubation is longer in direct proportion to the size of the animal and the consequent length of the nerves. Thus a mouse exhibits symptoms in eight to twelve hours, a guineapig in thirteen to eighteen, a rabbit in eighteen to thirty-six, while in man four days is the usual period, and in the horse five days. If an animal is injected with the poison in the nerve of a hinder limb, the spread of the poison to the important centres in the medulla can be prevented by section of the spinal cord. In this, as in the peripheral nerves, there appears to be a centripetal movement of protoplasm.

In cases of tetanus in man, the *incubation period* is very variable. Symptoms may appear in four or five days, although an incubation period so short as twenty-four hours has been recorded by Elver,<sup>3</sup> or they may be delayed for months. The more rapid the onset, the more acute are

<sup>1</sup> Permin, *Commun. de l'Inst. Sérother. de l'État Danois*, 1913, viii.

<sup>2</sup> *Arch. f. exper. Pathol. u. Pharmacol.*, 1903, Bd. xlix., Heft 6, p. 369.  
*Cf.* Marie and Morax, *Ann. de l'Inst. Pasteur*, 1902, xvi. 818; 1903, xvii. 335.

<sup>3</sup> *Deut. med. Woch.*, 1910, p. 961.

the symptoms and the more grave the prognosis. That spores of tetanus bacilli washed free from toxin and experimentally injected into healthy animals<sup>1</sup> may fail to germinate, or may circulate harmlessly until some injury is inflicted, when they multiply at the point of lesion and give rise to tetanus, has been shown by Canford;<sup>2</sup> and the long latency of some cases in the human subject may be explained on similar grounds.

**Toxins of the tetanus bacillus.**—The toxins of the bacillus may be obtained by growing the organisms in an atmosphere of hydrogen in broth from which all oxygen has been expelled by causing hydrogen gas to bubble through it. It is usual to add to the nutrient broth some reducing agent such as glucose, formate or sulphindigotate of soda, and then to tint the liquid with methylene blue to act as an indicator, the blue colour disappearing when the last trace of oxygen has been removed from the medium. Marie and Morax<sup>3</sup> state that a potent poison can be prepared by cultivating the bacilli in association with *Bacillus subtilis* in the presence of air.

The fluid obtained after about eight to ten days' growth is filtered through a porcelain filter, and the filtrate is ready for use. The poison gradually loses strength on keeping, probably owing to the formation of toxones similar to those described in connection with diphtheria (p. 103). Tetanus toxin is not destroyed by heating to 135° C. for ten minutes, but its action is slightly retarded; its potency is rapidly destroyed by sunlight. If the albuminous constituents of the culture-fluid are precipitated by alcohol or other reagents, the toxin is found in the precipitate; but it is probably not of an albuminous nature itself. Dean<sup>4</sup> concludes as follows with regard to it: "Tetanus toxin has thus many points

<sup>1</sup> *Centralbl. f. Bakt.*, I. Orig., 1907, xlv. 295.

<sup>2</sup> *Ibid.*, 1908, xlviii.

<sup>3</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 418.

<sup>4</sup> Art. "Tetanus" in "Quain's Dictionary of Medicine," edited by Montague Murray, 1902, p. 1688.



of resemblance to the soluble ferments; it is difficult to dialyse, is soluble in water, is precipitated by alcohol and tends to adhere to precipitates, is modified or destroyed by the action of air, sunlight, and comparatively low temperatures, and requires an incubation period for its action."

The mixed poison, as obtained from cultures, is almost certainly very complex in character. Tizzoni and Collina<sup>1</sup> state that it contains two toxins, one of which acts specially if it is administered subcutaneously, and produces convulsions, while the second becomes prominent in case of intravenous injection, giving rise to local tonic spasm. Ehrlich<sup>2</sup> has shown that there is also present a substance which produces hæmolysis (*tetanolysin*), and that an antilysin which neutralizes this is found in tetanus antitoxin.

Tetanus toxin, or *tetanine*, as it is called by French writers—using the word to apply to the poisons collectively—has a great affinity for the nervous system. If an animal has died of tetanus, the nerves leading from the seat of infection, and also the brain and spinal cord, contain the poison. The affinity of the brain tissue for the toxin has also been shown in another way by Wassermann and Takaki,<sup>3</sup> who found that if they made an emulsion of brain-substance, and mixed this with the toxins before injection into animals, no ill effects were produced. A protective influence was exercised even if the emulsion was injected at a different point from the toxin, without previous mixture. Cerebral substance seems thus to act as an antitoxin to the poison: in other words, the toxin has the power of combining with the side-chains of the cerebral cells; when, therefore, these cells are injected into another animal, they are capable of uniting with the free poison and so preventing it from attacking the living tissues of the animal.

The pathological effects of tetanus toxin are seen in the

<sup>1</sup> *Gaz. degli Ospedali*, 1901, No. 138.

<sup>2</sup> Quoted by Dean, *loc. cit.*

<sup>3</sup> *Berlin. klin. Woch.*, 1898, No. 1.

cells of the nervous system, and consist in swelling of the chromatic bodies and cell-body, followed by progressive chromatolysis. These lesions are of rather irregular distribution, being more uniform and intense in the brain than in the cord; and, with the possible exception of the early stages of the process, the changes are not specific of the disease.

The toxin exists in other organs besides the nervous system, and may be extracted from them by glycerin.<sup>1</sup> The peripheral nerves contain it, but have not the same neutralizing action as is possessed by the brain and spinal cord. The occasional appearance of a peripheral neuritis in patients who recover from tetanus has been attributed to a toxone analogous to that of diphtheria.<sup>2</sup>

#### TETANUS ANTITOXIN

**Preparation of antitoxin.**—For the practical preparation of antitoxin for tetanus, horses are used. These animals are very sensitive to the toxins of the disease, and great care is necessary in the process of immunization. At the beginning of the treatment, use is made of a toxin which has been attenuated by means either of some chemical agency (iodine trichloride is used by Behring) or by mixture with antitoxin, which is injected subcutaneously or intramuscularly in increasing doses at intervals of five to ten days. After the blood of the animal has been found to contain a considerable amount of antitoxin, as a result of these injections, the undiluted toxin may be administered. The method of procedure is the same as that already described in the case of diphtheria.

**Standardization of antitoxin.**—A method of standardizing tetanus antitoxin similar to that in use for anti-diphtherial serum has been introduced by Behring. A test toxin is prepared of such a strength that 0.01 c.c. will kill a guineapig of 500 grm. in about four days. This amount

<sup>1</sup> Waring, *Proc. Path. Soc. Lond.*; *Brit. Med. Journ.*, 1902, i. 965.

<sup>2</sup> Grünberger, *Wien. klin. Woch.*, 1904, p. 737.

of toxin is neutralized by  $\frac{1}{1000}$  of a unit of antitoxin. In other words, one unit of antitoxin will protect a guineapig against 1,000 minimal lethal doses of toxin.

According to Roux's method of standardization, the value of the antitoxin is expressed according to the number of guineapigs (calculated in grammes) which 1 c.c. of the serum will protect from a minimal fatal dose (for that number of guineapigs). Thus, if 1 c.c. of a certain serum will protect 100 guineapigs, each weighing 500 gm., against the minimal amount of toxin which would otherwise kill them in four days, this quantity of serum is said to contain 50,000 ( $100 \times 500$ ). This would be a very weak serum for use, 1,000,000 such units per cubic centimetre being an average strength.

Rosenau and Anderson<sup>1</sup> take as a unit of antitoxin ten times the least quantity of antitoxin which will keep a guineapig (of 350 gm.) alive for ninety-six hours after injection of an official toxic unit. This latter unit is the equivalent of 100 minimum lethal doses.

Unfortunately no antitetanic unit has gained universal acceptance in the description of the various serums on the market, the dose being generally calculated in cubic centimetres of serum, without any statement of the number of units contained. Hence cases recorded as treated with antitoxin have hitherto had very little value, owing to the impossibility of knowing how many units of antitoxin were really used. There is, however, at the present time a growing tendency, in all English-speaking countries, to adopt the U.S.A. antitoxic unit; and MacConkey,<sup>2</sup> who has recently compared the various units, states that the German (Behring) unit is equivalent to about 40 U.S.A. units; 1,600 Italian (Tizzoni) units are equivalent to one U.S.A. unit; 2 French (Roux) units are about equivalent to one U.S.A. unit; and the usual prophylactic dose of

<sup>1</sup> *Bull. No. 43, Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Service*, 1908.

<sup>2</sup> *Brit. Med. Journ.*, 1914, ii. 609; 1915, ii. 849.

10 c.c. of the Pasteur Institute serum is equal to about 600 U.S.A. units. In a considerable number of cases recorded in the past few years the number of units administered is stated; when, however, this important detail is missing, some idea of the amount injected can be arrived at if it is recollected that, according to its place of manufacture, 10 c.c. of antitetanic serum contains approximately 1,500 U.S.A., 500 to 1,000 Roux, 10 to 20 Behring, and 400,000 Tizzoni units.

**Experimental value of antitoxin.**—From experiments made in the laboratory, in which all the factors are under control, there can be no doubt that tetanus antitoxin, if given along with, or shortly after, a dose of the toxin, has the power of preventing the occurrence of the characteristic symptoms and of death. Dönitz<sup>1</sup> finds that, whereas a certain dose of antitoxin suffices to neutralize a definite quantity of the toxin when it is injected at the same time, if a space of four minutes is allowed to elapse between the administration of the toxin and that of the antidote, then a slightly larger amount of the latter is needed. If eight minutes intervene, then six times the original neutralizing dose of antitoxin is required; if sixteen minutes, then twelve times the dose; and at the end of one hour, twenty-four times the amount of antitoxin is requisite, and so on up to ninety-six hours. Thus, as in the case of diphtheria, the “mass” action of large doses of antitoxin may be exerted for a certain time after the poison is injected. This has been clearly shown by von Graff and Menschikoff,<sup>2</sup> who were able to remove the toxin from saturated liver-cells by soaking them in antitoxin; whilst Kraus and Amiradziti<sup>3</sup> further showed that the antitoxin does not penetrate the toxin-loaded cells, but that the toxin must diffuse out before it can be neutralized—the rate of the diffusion being accelerated by the presence of a high concentration

<sup>1</sup> *Deut. med. Woch.*, 1897, p. 430.

<sup>2</sup> *Centralbl. f. Bakt.*, I. Orig., 1912, lxi. 226.

<sup>3</sup> *Zeitschr. f. Immunitätsforsch.*, I. Orig., vi. 1.

of antitoxin in the surrounding fluid. But there comes a time when the toxin has entered so closely into combination with the cells that no amount of antitoxin is capable of withdrawing it. After that time even the minimal lethal dose is of necessity fatal.

Further, even if the blood of an animal is rendered antitoxic to tetanine, injection of this poison into the substance of its brain will still produce a fatal effect as surely as if no protective power had been gained.

**General considerations on the use of tetanus antitoxin.**—Tetanus is a comparatively rare disease in this country, and in times of peace is comparatively uncommon in most civilized parts of the world. Hence statistics are difficult to collect, and are generally founded on small numbers of cases, insufficient for the formation of accurate deductions. Further, the disease may occur in an acute or in a more or less chronic form, these varieties merging one into the other with no distinct dividing line; and in chronic cases, where the incubation period has been lengthy, the mortality is much less than in the acute. Owing to these peculiarities, it is difficult to calculate what the mortality from the disease was before the introduction of antitoxin; and the statistical method, such as was used for demonstrating the value of diphtherial antitoxin, is more liable to be vitiated by errors of observation.

In recorded cases of tetanus it is generally impossible to gather a definite opinion as to the severity of the case, and hence it is difficult to realize what would have been the chance of recovery without the use of antitoxin; while a comparison of a small series of six or eight cases with a theoretical general-mortality figure, of the vague description which we have just indicated, gives very little information. On the other hand, the observer's opinion in any individual instance, as to the effects produced by the remedy in abating symptoms or conducing to recovery, is unreliable.

The data obtained by means of experiments on animals, as to the behaviour of the poison in the body and as to the

neutralizing effects of the antitoxin, afford very clear indications as to what we may expect from the latter in the treatment of the disease. Nothing is more definitely established than that there comes a period of time at which the toxin is so closely attached to the cells of the nervous system that no amount of antitoxin will suffice to withdraw it or to counteract its effects. This usually occurs very early in the disease; so that the vital question that presents itself is, whether it is not already too late for the antitoxin to have any good effect when the disease has declared itself, i.e. when the symptoms, by which alone the infection can be recognized, have appeared. To this there can be but one answer. It is impossible to know in any given case whether the amount of toxin which has been taken up by the nerves is sufficient to cause a fatal termination. By administering antitoxin we cut off the supply of the poison and prevent further absorption. It might be that a quantity of toxin just short of the minimal fatal dose had been absorbed at the time when the case came under treatment. If the remedy were given at once, the further absorption of the poison would be stopped, and the balance would be turned in the patient's favour; whereas, if any delay in giving the remedy were allowed to arise, time might be afforded for the absorption of the additional amount of toxin necessary to cause death. Hence theoretical reasons lead us to the conclusion that the antitoxin should be used in all cases directly they come under treatment. No brilliant statistics of cures are to be looked for; probably only a few cases will be saved; but we can never know, till death has actually ensued, that the case before us is not one of the exceptions in which the use of antitoxin will turn the scale towards recovery.

#### **Statistics of the use of antitoxin in tetanus.—**

We have already pointed out that tetanus is too uncommon a disease for the statistics of it to be valuable; further, the use of antitoxin is of comparatively recent introduction, so that no large number of cases is available for the study of



its effects. The present European War has, it is true, unhappily supplied a considerable number of cases, the data concerning which may be regarded as adequate; but sufficient time has not yet elapsed for their classification and analysis. We are therefore still obliged to rely upon earlier statistics. Lambert<sup>1</sup> collected 262 cases, of which 151 recovered, giving a total mortality of 42·36 per cent. These were divided into 124 acute cases, of which 35 recovered, giving a percentage mortality of 71·77; and 138 chronic cases, of which 116 recovered, leaving a mortality of only 15·94 per cent. On these figures he pronounces strongly in favour of the use of the remedy, especially in the chronic cases, in which the previous mortality was about 40 per cent.; in these his figures show a reduction in mortality (due to antitoxin) of approximately 24 per cent. Abbe<sup>2</sup> saw 6 cases of tetanus in pre-antitoxin days, of which only 1 recovered; whereas of 9 others treated with this remedy 7 survived. Of these latter cases 5 were severe in type, and 3 of them recovered. For reasons already given it does not seem worth while to set forth in detail the figures given by different writers. Rozenraad<sup>3</sup> quotes the following records: Engelmann, 54 cases treated with antitoxic serum, mortality 28 per cent.; Lund, 167 cases, mortality 35 per cent.; and Kochler, 96 cases, mortality 34·4 per cent.

Busch<sup>4</sup> collected 147 cases with a mortality of 44 per cent. Schley<sup>5</sup> quotes Packard and Wilson's figures, viz. 1,216 cases with a mortality of 42 per cent., and Moschewitz's 461 cases with mortality 40·3 per cent. It is impossible to say how far these various collections of cases overlap one another.

**Mode of administration.**—*Subcutaneous* injection of

<sup>1</sup> *Op. cit.*

<sup>2</sup> *Ann. of Surg.*, 1900, xxxi. 273.

<sup>3</sup> *Quinzaine Thérapeut.*, 1904, p. 63.

<sup>4</sup> *Arch. f. klin. Chir.*, 1907, lxxxii., Heft 1.

<sup>5</sup> *N.Y. Med. Record*, 1904, p. 616.



antitoxin was that first employed in tetanus, as in diphtheria, and a large proportion of the cases on record have been treated by this method alone. It was, however, pointed out that the antitoxin was only slowly absorbed by this route, one or two days elapsing before the maximum concentration of antitoxin in the blood is reached; whereas it was important to neutralize the toxin, which had already got the start of the antidote, as quickly as possible. Hence *intravenous* injection certainly seems to be preferable to the subcutaneous route, as being speedier in its action—a question of minutes only.

Both of these methods effect a neutralization of the poison circulating in the blood, but they do not avail to counteract that which has already reached the nervous system. Two methods adapted to achieve this end, if it be possible in any way, have been recommended, namely, the injection of the antitoxin into the space between the dura mater and the brain (*subdural*) or spinal cord (*intrathecal*), and injection directly into the brain-substance (*intracerebral*). Each of these has its advocates, but for ease and safety of administration and generally for effectiveness the injection of the serum intraspinally after lumbar puncture is undoubtedly the best.

In favour of the *subdural* method, Leyden<sup>1</sup> points to the results of 11 cases which he has collected, among which there were 6 deaths and 5 recoveries. Two of the latter were cases of his own. It is certain that the cerebro-spinal fluid contains toxin in fatal cases of tetanus, and the subdural injection will neutralize this. It is, however, uncertain whether the toxin is taken up by the central nervous system directly from the fluid in which it is bathed. Meyer and Ransom, as already quoted, do not think that it is; but there is no doubt that other substances, such as cocaine, are thus absorbed. Further experiment seems necessary in order to decide this question positively.

<sup>1</sup> *Deut. med. Woch.*, 1901, No. 29, p. 477.

Jacob<sup>1</sup> states that he was successful in saving two-thirds of his cases at the Charité Hospital in St. Petersburg which were treated by the subdural method. He withdraws 10 c.c. of cerebro-spinal fluid by lumbar puncture, and then slowly injects 10 to 20 c.c. of antitoxin. Penna<sup>2</sup> gave the remedy beneath the cerebral membranes, using large quantities of serum—in one case 60 c.c. at a dose. Daily injections were given, amounting in all to 100–240 c.c. per case. Of 5 patients, 3 recovered and 2 died—the latter of intercurrent pneumonia, not of tetanus. Wallace and Sargent<sup>3</sup> report 4 cases treated by injection into the spinal theca; of these 3 recovered, 1 being a severe case and 2 subacute.

Hoffmann<sup>4</sup> contrasts 13 cases treated by subcutaneous injection only, with 7 deaths, with a further 16 cases (including extremely severe cases with short incubation periods) treated by intraspinal injections, with only 2 deaths.

Theory is very strongly in favour of the method of *intracerebral* injection. It has been pointed out that, if the toxin is injected directly into the brain, no amount of antitoxic power in the blood will avert a fatal issue. The toxin appears to be passed on from one cell to another in the central nervous system, and it is to be supposed that the antitoxin will also be thus diffused. Roux<sup>5</sup> saved 35 out of 45 guineapigs inoculated with tetanine by this method, whereas by the subcutaneous injection he had only 2 recoveries out of 17 animals. Letoux<sup>6</sup> records 4 cases of recovery in human patients after intracerebral injection.

<sup>1</sup> Rousski *Vratch*, Jan. and Feb., 1902 (abstr. in *Journ. of the Amer. Med. Assoc.*, 1902, i. 977).

<sup>2</sup> *Semana Medica*, Oct. 31, 1901 (abstr. in *Journ. of the Amer. Med. Assoc.*, 1902, p. 602).

<sup>3</sup> *Lancet*, 1904, i. 642. Cf. Neugebauer, *Wien. klin. Woch.*, 1905, No. 18.

<sup>4</sup> *Beitr. z. klin. Chir.*, Oct., 1907, lv.

<sup>5</sup> Quoted by Abbe, *op. cit.*

<sup>6</sup> *Semaine Méd.*, 1901, p. 349.

He administered 10 c.c. of antitoxin into each hemisphere. Abbe<sup>1</sup> also speaks in favour of the intracerebral route.

Bruce,<sup>2</sup> on analysing 215 cases of tetanus treated in the home military hospitals, came to the conclusion that intrathecal injections, alone or combined with intravenous and subcutaneous injections, of antitetanic serum gave the lowest mortality.

*Intraneural* injection has also been practised, in view of the supposed route by which the poison is absorbed. Good results are recorded in individual cases.<sup>3</sup> It is best to make use of the subdural method in addition.

In a recent communication Ashhurst and John<sup>4</sup> advise the simultaneous use of several modes of administration of the serum. They expose the nerves leading from the wound by surgical incision, and inject into them, as near to the spinal cord as possible and towards this structure, as much serum as can be introduced. They then give intrathecally 3,000 units of the antitoxin, and the same quantity or twice as much into the muscles around the wound, while 10,000 units are administered intravenously. The intraneural and intrathecal injections are repeated daily, and the intravenous dose may also be repeated if desired. Coincidentally the wound is carefully cleaned up, and chloral and bromide are given by mouth or rectum to diminish the irritability of the spinal cord.

Behring<sup>5</sup> considers that no good results are to be hoped for from the use of antitoxin if it is administered more than thirty hours after the onset of symptoms, or if less than 100 units (on his system) are given. According to the experience of Moeller,<sup>6</sup> even if these postu-

<sup>1</sup> *Op. cit.*

<sup>2</sup> *Brit. Med. Journ.*, 1915, ii. 593.

<sup>3</sup> See Rosenraad, *Quinzaine Thérapeut.*, 1904, p. 63; Apert and L'Hermitte, *ibid.*, 199; Sicard, *ibid.*, 199; Clairmont, *Wien. klin. Woch.*, 1905, No. 49; Rogers, *Med. Record*, 1904, lxx. 813, lxxi. 12.

<sup>4</sup> *Amer. Journ. Med. Sci.*, 1913, cxlv. 806, clxvi. 77.

<sup>5</sup> *Deut. med. Woch.*, 1900, No. 2.

<sup>6</sup> *Ibid.*, 1901, No 47, p. 814.

lates are fulfilled, no great fall in the mortality is to be looked for.

A considerable number of isolated cases are reported, but as they are by different observers, using different brands of serum and employing a variety of methods, little would be gained by tabulating them. Tizzoni<sup>1</sup> considers that the serum which he prepares is superior to that of Behring, while German writers<sup>2</sup> apparently hold that the disease in Italy is less virulent than in their own country. No exact data seem to be available for estimating the truth of these international amenities.

On the whole, as was to be anticipated on the theoretical grounds already set out, the curative treatment of tetanus by antitoxin is disappointing, as compared with the results obtained in diphtheria. Nevertheless, it is our duty to give the remedy a trial in all cases, since it is possible that it will do good, and it can in any case do practically no harm.

**Dose of antitoxin.**—As already mentioned, records of cases generally state the number of cubic centimetres of serum injected, and not the number of units. A dose is usually from 10 to 20 c.c. ; and although the amount of antitoxic units contained in the serum is not often noted on the bottle, and hence is not known to the administrator, it may be gauged by reference to the equivalents given on p. 144. Behring advises that not less than 100 of his units (4,000 U.S.A. units) should be given at once. Amounts of 10 to 20 c.c. are easily administered subdurally, around either the spinal cord or the brain, or into the lateral ventricles. From 3 to 5 c.c. can be injected into the substance of the brain by slow instillation. Amounts up to 50 or 100 c.c. can be given, if necessary, intravenously. The important point, however, is that the injection must be given boldly and in adequate dose, and the dose must be repeated frequently.

**Prophylactic use of antitoxin.**—There is ample

<sup>1</sup> *Riforma Medica*, 1901, i. 366.

<sup>2</sup> See Pfeiffer, *Zeitschr. f. Heilkunde*, Bd. xxxiii., Heft 2.

evidence that antitoxin proves of greatest service in the prevention of tetanus. Experiments on animals point strongly in this direction. Actual results are also encouraging. In veterinary practice some striking statistics are quoted by MacFarland.<sup>1</sup> In one of the large factories for the production of antitoxin (diphtherial) much trouble was at one time caused by the incidence of tetanus among the horses. At last systematic use of prophylactic injections of antitoxin was instituted, and the result was that, whereas in one year (1898) the death-rate among the animals was 10 per cent., in the year following it fell to 1 per cent., and in the year after that to a mere fraction. Nocard<sup>2</sup> reports concerning a certain district that of 2,708 horses injected with antitoxin none developed tetanus, although among other horses in the same part of the country there were 220 cases of the disease. Vaillard<sup>3</sup> adds 13,124 in the practice of eight veterinary surgeons, between the years 1898-1906, treated prophylactically with tetanus antitoxin without a single case of tetanus occurring, and notes that two neighbouring veterinarians saw 139 cases of tetanus in other practices during the same period. Herhold,<sup>4</sup> who was surgeon to the German contingent on the expedition to Peking, at first lost several patients by tetanus. Afterwards he used prophylactic injections in all cases of crushed wounds contaminated with dirt, and no more cases of the disease occurred. Fisch<sup>5</sup> considers that the preventive use of the antitoxin was of considerable service in the epidemic which occurred at St. Louis from contaminated diphtherial antitoxin.

The records of Independence Day in the United States afford the best examples of the results of the systematic use of prophylactic doses of antitoxin. Each celebration

<sup>1</sup> *Journ. of the Amer. Med. Assoc.*, July 4, 1903, p. 34.

<sup>2</sup> Quoted by Vaillard, "*Médicaments Microbiens.*" Paris, 1912.

<sup>3</sup> *Op. cit.*

<sup>4</sup> *Deut. med. Woch.*, 1901, No. 30, p. 479.

<sup>5</sup> *Interstate Med. Journ.*, Dec., 1901.

is responsible for over 4,000 cases of dirty and contused wounds, which are followed in many instances by tetanus; and in 1903 there were 406 deaths from this disease. Since 1904 antitoxin has been extensively used, and, although the numbers of the wounded have not appreciably diminished, there has been a consistent fall in the mortality from tetanus, as will be seen by the following table :<sup>1</sup>—

## STATISTICS OF INJURIES, INDEPENDENCE DAY, U.S.A.

YEAR	DEATHS		INJURIES		TETANUS DEATHS PER 1,000 INJURIES
	TETANUS	OTHER DISEASES			
1903 . .	406	60	3,983 . .		102
1904 . .	91	92	3,986 . . .		23
1905 . .	87	95	4,994 . . .		17
1906 . .	75	83	5,308 . . .		14
1907 . .	62	102	4,249 . . .		14
1908 . .	55	108	5,460 . . .		10
1909 . .	125	90	5,092 . . .		24
1910 . .	67	64	2,792 . . .		24
1911 . .	10	47	1,546 . . .		6
1912 . .	6	35	947 . . .		6
1913 . .	3	29	1,131 . . .		3

Although statistics are not yet available, the experience of the surgeons employed on the Western front during the first year of the present European War leads them to express a high opinion of the value of antitetanic serum used prophylactically.

At the same time, it seems necessary to admit that an injection of antitoxin is not a certain preventive of tetanus, as Reynier<sup>2</sup> records that in a hospital in Paris, in consequence of an outbreak of the disease, a patient who had received a prophylactic injection nevertheless developed

<sup>1</sup> *Journ. of the Amer. Med. Assoc.*, Sept., 1912, and Sept., 1913.

<sup>2</sup> Quoted by Moeller, *Deut. med. Woch.*, 1901, No. 47, p. 814.

tetanus. She finally recovered, whereas two other patients died of the disease; so it is possible that even here some good was done, and what would otherwise have been a fatal attack was rendered milder. Walther<sup>1</sup> records the case of a German soldier wounded by shrapnel, who received 10 c.c. antitoxin as a prophylactic, but developed tetanus the following day.

We may conclude that, in countries where tetanus is a comparatively common complication, all cases of crushed or dirty wounds should receive a prophylactic injection of antitoxin; and American experience would place the prophylactic dose at from 500 to 1,500 or more U.S.A. units, according to the length of time that has elapsed since the infliction of the injury. How long the protection will last is not known. As the serum, being derived from the horse, is exactly analogous to diphtherial antitoxin, it seems legitimate to assume that it will be excreted or neutralized at the same rate as the latter: hence we may conclude that the protection will remain effective for about three weeks; and Rowan<sup>2</sup> records a case where protection for a period of twenty-four days was obtained from a dose of 1,500 U.S.A. units. Dehne and Hamburger,<sup>3</sup> however, believe that it ceases after about one week; hence the French practice of repeating the prophylactic dose at the end of seven to ten days is sound. Levin<sup>4</sup> is of a similar opinion, and therefore advocates a series of relatively small doses rather than one large one.

**Ill effects of antitoxin.**—The same by-effects may be expected to occur after the use of tetanus antitoxin as after antidiphtherial serum, since it is horse's serum which is used in both cases. Di Gaspero<sup>5</sup> describes a case in which a fatal issue occurred, due, as he thinks, to serum;

<sup>1</sup> *La Presse Méd.*, 1914.

<sup>2</sup> *Wien. klin. Woch.*, 1907, Nos. 1-3.

<sup>3</sup> *Journ. Med. Assoc.*, 1910, p. 533.

<sup>4</sup> *Zeitschr. f. Immunitätsforsch.*, 1909, i. (Orig.) 3.

<sup>5</sup> "Die Therap. der Gegenwart," 1902, p. 139.



but the accuracy of this inference is very doubtful. Rashes of various kinds—scarlatiniform, bullous, urticarial, etc.—are also recorded.

### TREATMENT WITH CEREBRAL EMULSION

In view of the great affinity of the substance of the central nervous system (brain and spinal cord) for tetanus toxin, use has been made of emulsion of fresh brain-substance as treatment for the disease. Krokiewicz<sup>1</sup> recorded 16 cases in which use was made of this preparation, an unfiltered emulsion being injected hypodermically. Of the 16 cases, 13 recovered, 3 of them being severe attacks.

When we consider the difficulty with which an emulsion of cerebral substance must be absorbed and reach the circulation, it is easy to realize that the curative action of such a remedy must necessarily be slower than that of antitoxic serum. The latter should therefore have the preference, if it be available. If, however, no antitoxin be at hand, it would certainly be not only legitimate but also advisable to make trial of cerebral emulsion. The mode of action of the two remedies is theoretically the same, the receptors of the cerebral cells being available in the emulsion to anchor the toxin and neutralize it, just as the free receptors (side-chains) do in the antitoxin. The diffusion of the emulsified cells must be less rapid than that of the cast-off receptors—which are presumably separate molecules, not whole cells.

### CONCLUSIONS

1. Tetanus antitoxin cannot be expected to *cure* any large proportion of patients in whom the disease has developed, but it should be given in all cases of tetanus, since it may just turn the scale in the patient's favour by neutralizing the poison circulating in the blood, although it may not reach that already absorbed by the nervous system.

<sup>1</sup> *Klin. therap. Woch.*, Feb. 8, 1903.

2. **Curative dose.**—In all cases, intraspinal injection through a lumbar puncture (of high-valency serum equal in volume to the amount of cerebro-spinal fluid that can be first withdrawn—at least 5,000 U.S.A. units, or the equivalent in Continental serums) should be practised, immediately followed by intravenous injection of at least 5,000 U.S.A. units. These injections may be repeated as often as seems necessary, at intervals of six to twelve hours.

3. Intracerebral injection possibly offers the best chance of neutralizing the poison already absorbed, and may be used in very severe cases, intravenous or subcutaneous injections being employed at the same time.

4. **Prophylactic dose.**—In countries where tetanus is common, prophylactic injections of antitoxin should be used in all cases of crushed wounds, especially those which have been soiled by dirt or other foreign matter, and in deep incised wounds similarly contaminated. The antitoxin may be given subcutaneously or intramuscularly in the vicinity of the wound, or intravenously.

5. The prophylactic dose may be stated as 500 to 1,000 U.S.A. units, the latter being an ample dose if used immediately. If, however, any appreciable interval has elapsed since the receipt of the injury, this latter dose must be doubled, trebled, or quadrupled, and the serum must be injected *before the surgical toilet of the wound is undertaken*, to obviate the danger of opening up fresh areas for the absorption of unneutralized toxin; for this reason, too, a large prophylactic dose of serum must be injected *intravenously* immediately before any extensive operation is commenced.

6. The prophylactic dose may be repeated a week later with advantage; in severe wounds the dose should certainly be repeated once or twice at intervals of seven days.

## CHAPTER VII

### SNAKE-BITE

**Classification of snakes.**—The poisonous varieties of snakes belong chiefly to the families Colubridæ and Viperidæ. The best-known kinds which belong to the Colubrine group are the Cobras (*Naja*), the Coralline snakes (*Elaps*), the Kraits (*Bungarus*), and the Death-adder (*Notechis*). To the Viperine division belong the Common Viper (*Vipera berus*), Russell's Viper (*Daboia*), the Puff-adder (*V. arietans*), the Rattlesnakes (*Crotalus*), the Bush-master (*Lachesis*), the Tiger-snake (*Hoplocephalus*), and the Copperhead (*Denisonia*). The poisonous water-snakes (*Hydrophinae*) are classed as Colubrine snakes. The classification depends mainly on small differences in the teeth and the bones of the head.<sup>1</sup>

The amount of poison injected in an individual case depends on the efficiency with which the stroke was delivered, while the virulence of the poison varies with the health and vigour of the snake at the moment, and to some extent with the season of the year. Calmette, using cobras that had been kept in captivity for some time, believed that a full-sized cobra would inject at one stroke about 30 to 45 mg. of venom; but Cunningham puts the quantity at 254 mg., and Rogers at 249 mg. Lamb, dealing with freshly-caught cobras, reckons the average amount as 200 mg.<sup>2</sup>

Different animals vary somewhat in the degree of suscep-

<sup>1</sup> An account of the varieties of poisonous snakes may be found in Calmette's "Venoms, Venomous Animals, and Antivenomous Therapeutics," trans. by Austin; London, 1908.

<sup>2</sup> See Rogers, *Ind. Med. Gaz.*, Sept., 1904, p. 332; Lamb, *Glasgow Med. Journ.*, Feb., 1903, p. 85.

tibility which they present to the action of snake-poison. Rabbits, guineapigs, and herbivorous animals in general seem more susceptible than carnivora, such as the cat or the dog. Man is probably about equal in this respect to the latter class. The minute dose of poison which is capable of causing symptoms is illustrated by the experience of the late Mr. Frank Buckland, who accidentally pricked his finger in the process of dissecting the body of a rat killed by the bite of a poisonous snake. Only an infinitesimal amount of fluid could thus have entered his system, and the original dose of poison had been diluted by diffusion throughout the body of the animal; nevertheless, alarming symptoms of faintness and collapse ensued.

**Nature of the poison.**—The poison may be collected by killing the snake and dissecting out the gland and receptacle; or the snake may be “milked” by pressure on the sac; or it may be made to bite on a watch-glass covered with an india-rubber membrane, and the poison thus collected in the glass. The last method is probably the best. The poison is dried *in vacuo*, and may be preserved indefinitely in this condition.

It has long been known that the poisons of different snakes are not identical in their effects; the poisons of the viperine group having, as a rule, a more intense local action, those of the colubrine family rapidly producing a general intoxication. Calmette, however, to whom we are indebted for the original preparation of an anti-venomous serum (*antivenene*), considered that the main toxin, at all events, was the same in all venoms, and could be counteracted by one and the same antitoxin. This view was combated by Martin, who, as the result of his experiments, came to the conclusion that there were two substances present in the venom of all snakes, varying, however, in proportions in different species. One was a globulin, coagulable by heat; the other a peptone, which was resistant to it. The former is responsible for the general nervine intoxication, the latter for the local effects and blood-changes.

Lamb<sup>1</sup> has given reasons for rejecting these views. He is inclined to think that the poison of each genus of snakes is to some extent specific, containing a kind of poison not met with in other varieties. Thus he holds that though the poisons of the cobra and the daboia are each complex, no single constituent is common to both. The reasons for this will be clearer when we come to consider the action of antitoxic serum as an antidote to the poisons of different snakes. In a later article, written with C. J. Martin,<sup>2</sup> the statement is made as the result of a study of many venoms and antivenenes, that "antivenomous serums are highly but not strictly specific." Lamb originally considered that in all probability the poisonous element was not proteid in nature. He showed that the proteins in the different venoms are, as far as can be determined, the same in all, while the effects produced are diverse. This would bring snake-poison into the same category as the toxins of diphtheria and tetanus, of which the former was shown to be developed in a non-albuminous medium, while the latter, according to Dean, is also of the nature of a ferment rather than an albumin. Lamb<sup>3</sup> appears, however, to have changed his opinion and now believes in the proteid nature of the poison. Faust,<sup>4</sup> on the other hand, states that he precipitated a poison (*ophiotoxin*), five times as toxic as crude venom, from cobra-venom by means of alcohol after all the proteins had been removed.

**Action of venom.**—Locally, the effects produced by snake-bite are swelling, redness, and ecchymosis; if the patient survive the first effects, cellulitis and sloughing of the parts may occur, and the œdema may spread up the

<sup>1</sup> "Scientific Memoirs by Officers of the Med. and Sanit. Depts. of the Govt. of India," No. 5, "Specificity of Anti-venomous Sera," 1903.

<sup>2</sup> Allbutt and Rolleston's "System of Medicine," vol. ii., Part 2, 1907, p. 815.

<sup>3</sup> *Glasgow Med. Journ.*, Feb., 1903, p. 86.

<sup>4</sup> *Arch. f. exp. Path.*, 1907, lvi. 236.

affected limb. The general effect of the poison is shown in its action upon the nervous system, which takes the form of depression, collapse, nausea or vomiting, incoördination, paralysis, and convulsions, ending in coma and death by asphyxia or heart-failure. Hæmorrhagic discharges from mucous membranes are sometimes seen.

Experimentally, snake-poison is found to have a solvent action on the red corpuscles of the blood (hæmolysis). Lamb finds that both cobra- and daboia-venom have this property. Cobra-poison, however, acts more powerfully outside the body than within it, while daboia-venom is more hæmolytic *in vivo* than *in vitro*. The hæmolytic substance in cobra-venom is not coagulable by heat. According to Harnack and Hildebrand,<sup>1</sup> the venom of the rattlesnake (*crotalotoxin*)<sup>2</sup> acts almost instantaneously when administered intravenously, but more slowly when injected into a muscle. Small repeated doses produce wasting, and a waxy degeneration of the muscle-fibres is seen after death. Rattlesnake-venom is attenuated by heat and by the action of hydrochloric acid. Cobra-hæmolysin is also weakened by heat, but recovers its virulence on standing.<sup>3</sup> Flexner and Noguchi<sup>4</sup> assert that the hæmolytic agent in venom is of the nature of a copula or intermediary body (*see* p. 8). The complement which is necessary to complete its action on the blood-corpuscles is contained within the corpuscles

<sup>1</sup> *Münch. med. Woch.*, 1912, p. 1426.

<sup>2</sup> Crotaline, or rattlesnake-venom, has been used for medical treatment, especially in epilepsy, for which Mays advises doses of  $\frac{1}{200}$  to  $\frac{1}{100}$  grain, given every fourth day. The same writer believes this substance to be useful in chronic pulmonary tuberculosis: he makes a solution of 1 gr. in a mixture of 100 min. of glycerin and 400 of water, giving doses of  $\frac{1}{200}$  to  $\frac{1}{100}$  gr. into the "lung cavity" (? substance of the lung). The solution may also be used as a nebula (*Med. Record*, 1913, lxxxiii. 561). *See also* Spangler, *New York Med. Journ.*, Sept. 9, 1911; and Fackenheim, *Münch. med. Woch.*, Sept. 5, 1911.

<sup>3</sup> *See* Flexner and Noguchi, *Journ. Med. Research*, 1904, vi. 363; Morgenroth and Pane, *Biochem. Zeitschr.*, 1906, i. 354.

<sup>4</sup> *Univ. of Pennsylvania Med. Bull.*, 1902, xiv. 438, and xv. 345.

themselves (endo-complement). This view has been confirmed by Kyes,<sup>1</sup> who was successful in preparing a chemical compound resulting from the union of the copula and lecithin. This he named "cobra-lecithide." It has a direct hæmolytic action. Snake-poison also possesses the power of agglutinating red corpuscles, the clumps thus formed being disintegrated again by the action of permanganate of potassium. The agglutinative power is removed by heating the venom to 75° or 80° C., whereas the hæmolytic power still remains after this treatment, showing that the two properties are dependent on distinct toxins. Leucocytes are destroyed by the poison as well as red corpuscles (leucolysis). The different kinds of leucocytes contained in rabbit's blood are unequally affected by the leucolytic substance, the lymphocytes being the least susceptible.

The ecchymoses seen in cases of snake-bite are due to the action of the poison on the endothelial cells forming the lining membrane of the capillaries. This action is analogous to hæmolysis, and is due to a destructive "lysis" of these cells by a special substance, which is called by Flexner and Noguchi "hæmorrhagin," from its effect in producing hæmorrhages. It is suggested by these observers that possibly similar substances may exist in the blood of patients suffering from the various forms of purpura. These authors further state that venom has the power of dissolving other cells besides blood-corpuscles (e.g. liver-cells, spermatozoa, etc.). Daboia-venom also liquefies gelatin by means of a special ferment.

According to Lamb<sup>2</sup> the venom of the daboia produces intravascular clotting of the blood, whereas cobra-poison, on the other hand, has an exactly opposite effect, causing a diminution in the coagulability of the blood. Death in cases of bite by the daboia results from the extensive character

<sup>1</sup> *Berlin. klin. Woch.*, 1903, Nos. 42, 43.

<sup>2</sup> "Scientific Memoirs of the Government of India," No. 4, 1903, "On the Action of the Venoms of the Cobra (*Naja tripudians*) and of the Daboia (*D. Russellii*)," etc.



of this clotting. Curiously enough, it appears that a small dose of the poison, insufficient to cause this phenomenon, is followed by a diminution of coagulative power; and if this has once been produced, no subsequent injection of further doses of the poison will any longer produce clotting (*cf.* antianaphylaxis). This peculiar phenomenon is not reproduced *in vitro*, and is dependent on some obscure vital action. The substance in cobra-venom which reduces the coagulative power of the blood is not a protein coagulable by heat. The addition of snake-venom to blood has the effect of reducing the bactericidal power of the latter by depriving it of the necessary complements, which become fixed to the copulas present in the venom. The latter does not contain complement.

The element in venom which acts on the nervous system is called by Flexner and Noguchi "neurotoxin." It, too, is of the nature of a copula, and acts by fixing a suitable complement to the nerve-cells. These observers also found that the brain was the organ in the body which contained the most neutralizing substance for venom, i.e. that its cells have the greatest affinity for the toxin. An animal injected with a minimal lethal dose of venom mixed with emulsion of brain lived many hours longer than one which received the same quantity of poison in blood-serum or similar fluid. After death acute degeneration of the nerve-cells (chromatolysis) is found (Hunter).<sup>1</sup> Rogers<sup>2</sup> states that the poison of the sea-snake, *Enhydrina*, has an action resembling that of curare.

The principal poisonous substances in snake-poison are therefore (1) hæmolytic, (2) leucolytic, (3) hæmorrhagic, and (4) neurotoxic. The bodies having these separate actions are probably different in the various kinds of poisonous snakes. Hence it is evident that the term "snake-poison" or venom includes a very complex group of chemical substances.

<sup>1</sup> *Glasgow Med. Journ.*, Feb., 1903, p. 98.

<sup>2</sup> *Lancet*, 1904, i. 349.

## ANTIVENENE

**Preparation of antivenene.**—The possibility of preparing an antitoxic serum (*antivenene*) for the treatment of snake-bite was first practically demonstrated by Calmette, of Lille. His serum is manufactured by injecting a horse with gradually increasing quantities of a mixed venom, containing 80 per cent. of cobra-poison and 20 per cent. of viperine venom. The mixture is heated before the injections are given, as the crude poison is so intensely toxic that the horses are often killed by the minute quantities used for immunization. Thus MacFarland<sup>1</sup> states that he lost two out of three horses on which he practised the inoculations. It is therefore necessary to proceed with the greatest caution in these injections. A further difficulty is met with in the process, owing to the need of procuring large quantities of venom for the later injections—a need which is not easily satisfied, for obvious reasons. It is advisable to administer to the horse before inoculation one or more protective doses of antivenene, in order to enable it to withstand the first injections of the poison. Otherwise the method adopted is practically the same as that already described for the antitoxins of tetanus and diphtheria. Tidswell<sup>2</sup> took more than three years in immunizing a horse against the venom of the Australian tiger-snake, owing to a combination of the above-mentioned difficulties.

**Action of antivenene.**—Calmette claims that the antivenene which he prepares is capable of neutralizing the effects of the venom of any snake, whatever the species to which it belongs. The mixture of venoms which he uses for the inoculation is designed to render the horse resistant to the poisons of viperine as well as colubrine snakes, even if it be not the case, as he apparently holds, that the venoms of all kinds of snakes are identical. It has, however, been

<sup>1</sup> *Journ. of the American Med. Assoc.*, 1901, xxxvii. 1597.

<sup>2</sup> *Australasian Med. Gaz.*, April 21, 1902.

pointed out by Hanna and Lamb<sup>1</sup> that the heating process to which the mixed poison is subjected before injection, in order to render it less virulent, is capable of destroying the potency of viperine poison altogether, or almost entirely. Hence only the cobra-venom is actually left to immunize the horse.

Experience seems to confirm this view to a great extent. Thus Tidswell finds that Calmette's serum has no effect in neutralizing the poison of the Australian tiger-snake (*Hoplocephalus curtus*); nor had an antivenene prepared from the venom of this snake any antidotal power against the poisons of other snakes met with in the same continent (*Echis*, *Notechis*). Lamb similarly found that cobra-antivenene has no antitoxic power against the bite of the daboia; and in a later memoir he pointed out that it was unavailing against the poisons of the snakes known as *Bungarus coruleus* and *Echis carinata*. Serum prepared with the venom of the hoplocephalus by Tidswell had no neutralizing effect upon the venom of bungarus, cobra, or daboia, and an anticrotalus serum failed to influence the course of symptoms produced by the venom of other snakes (Noguchi). On the other hand, Körbel<sup>2</sup> found antivenene useful in the bites of vipers (*V. aspis*, *V. berus*, *V. ursini*, *V. ammodytes*), and Rogers<sup>3</sup> found that it neutralized the venom of the king-cobra (*Hamadryas*), of the krait (*Bungarus*), and of the sea-snake (*Enhydrina*).

These results are of considerable practical importance. If the venoms of the different snakes are thus specific in nature, so that a serum prepared from one of them has no neutralizing effect on poison derived from another species, the question of the practical therapeutics of snake-bite becomes much less simple than was originally hoped by Calmette. It would seem necessary to have at hand in all cases a supply of serums for all the different varieties of

<sup>1</sup> *Journ. of Pathol. and Bacter.*, 1902, viii. 1.

<sup>2</sup> *Wien. med. Woch.*, 1908, p. 399.

<sup>3</sup> *Indian Med. Gaz.*, Sept., 1904, p. 332.

snakes found in any district, or else to prepare a polyvalent serum by injections of the poisons of all of these reptiles. It does not seem to be known at present how far the latter suggestion is feasible. On the other hand, it is evident that if separate serums were prepared for each kind of snake, it would often be necessary to inject all of them in a case of snake-bite, since the patient could not be expected to know what was the kind of snake which bit him. Bites from snakes often occur at night, when it would be impossible for anyone to identify the assailant; while those who are not skilled zoologists would in any case not be likely to know one poisonous variety from another.

With regard to the neutralizing action of antivenene on the different constituents of snake-poison, there seems some divergence of opinion. Flexner and Noguchi<sup>1</sup> state that antivenene is capable of inhibiting the effects, not only of the neurotoxin or main poisonous element, but also of the hæmolytic and other materials. On the other hand, MacFarland<sup>2</sup> finds that it is very difficult, if not impossible, to produce immunity to the local irritation of the poison. He considers, however, that the remedy should be used in all cases, as the counteraction of the most deadly toxin allows the body to concentrate all its resisting powers on repelling the local irritant. According to Auché and Vaillant-Hovius<sup>3</sup> the presence of antivenene does not prevent hæmolysis altogether, but renders it less intense and more transitory. If the neural toxin of snake-poison be removed, the body which gives rise to coagulation of the blood may still cause death, if it is present in a sufficient amount.

Noguchi<sup>4</sup> has prepared an antivenene against rattlesnake-bite (antierotalus serum), and Vital<sup>5</sup> prepares two serums,

<sup>1</sup> *Journ. Exper. Med.*, 1902, vi. 277.

<sup>2</sup> *Op. cit.*

<sup>3</sup> *Archives de Méd. Expérimentale et d'Anatomie Pathol.*, 1902, xiv. 221.

<sup>4</sup> *Univ. Penna. Med. Bull.*, Aug., 1904, p. 154.

<sup>5</sup> Quoted by Alabrese, *Abstr. Centralbl. f. inn. Med.*, 1907, p. 152.

one against crotalus and the other against bothrops, and mixes them to form a polyvalent remedy.

**Standardization of antivenene.**—For the purpose of experimental study of the action of venom and of antivenene on animals it is necessary to arrive at some standard of virulence and of protective power respectively. This is done by determining the minimal lethal dose for a certain kind of animal (rabbit or rat), calculating the weight of the animal in grammes. Thus Lamb found that 0.05 mg. (0.00005 grm.) of the venom of *Echis carinata* per kilogramme of body-weight was fatal to rabbits; in other words, a rabbit weighing 1,000 grm. would be killed by the above quantity of poison, while one weighing 1,500 grm. would require half as much again. Of the venom of *Bungarus fasciatus* 0.7 mg. was required to produce the same effect. The venoms of different snakes thus differ markedly in their actual toxicity. Further, as was previously stated, different species of animals vary somewhat in their susceptibility to snake-poison, while the actual toxins are probably very different in the various kinds of snakes. Hence no accurate measurement of toxins and antitoxins applicable to all can possibly be arranged.

The only antitoxic serum on the market is Calmette's antivenene, which is effective for cobra-poison. This is standardized by experiments on rabbits. The amount of serum which will protect a rabbit weighing 2,000 grm. against the smallest amount of the toxin that would otherwise kill it is said to contain 2,000 units of antitoxin. The Pasteur Institute of India also issues an antitoxic serum, which is twice as strong as Calmette's. The whole matter is as yet in so experimental a stage that the standardization of the antivenene is scarcely of practical therapeutic importance.

**Dose of antivenene.**—Doses of 10 to 30 c.c. are recommended by Calmette. Lamb, however, considers that this amount of a serum of the ordinary strength is not sufficient to protect against the whole amount of poison which a full-

grown, healthy snake can inject at a bite, and he advises the use of not less than 100 c.c., if this quantity be available. Rogers calculates that as much as 400 to 800 c.c. may be required. If any time has elapsed since the bite, the remedy should be given intravenously. If the case be seen at once, injection into the neighbourhood of the bite may be employed. Ordinary measures, such as the constriction of the bitten limb by a tight ligature above the seat of injury, pressure to squeeze out any poison lying free in the punctures, and stimulating remedies, must not be omitted.

### CONCLUSIONS

1. Calmette's antivenene should be used promptly in all cases of snake-bite. It protects effectually against cobra-bites. It probably has little effect against the venom of snakes belonging to other genera; but this matter is still under investigation, and in any case it is difficult, if not impossible, to be certain of the kind of snake which has inflicted the bite in countries where several kinds are met with.

2. The dose should be 100 c.c. or more, if possible, and should be injected intravenously as soon as possible after the bite.

3. Fuller investigation is necessary with regard to the manufacture of a polyvalent serum applicable to the bites of more than one kind of poisonous snake.

## CHAPTER VIII

### HYDROPHOBIA (RABIES)

**Causation.**—Up to the present time the actual cause of hydrophobia, or rabies as it is called when it affects the lower animals, is absolutely unknown. A large number of organisms have at one time and another been announced as the excitants of the disease (bacteria, protozoa, etc.), but no one of them has so far withstood the test when its claims were more fully investigated.

In 1903 Negri<sup>1</sup> discovered certain bodies in the large nerve-cells of the cerebral cortex, cerebellum, etc., which he maintained represented one phase of the life-cycle of a specific parasite. Some of the organisms contain a number of small refringent bodies like spores. They appear, as a rule, just before the onset of symptoms in the rabbit, and were found in one case of human hydrophobia. Negri regarded the bodies as protozoa, and stated that they occurred only in hydrophobia and not in other conditions. They are easily demonstrable by ordinary staining methods. Noguchi<sup>2</sup> believes that he has observed multiplication of these parasites in artificial media, and confidently asserts that they are the causal agents of the disease; but Volpino,<sup>3</sup> repeating Noguchi's experiments, declares that the multiplication forms are merely droplets of lipid—and there the matter rests for the moment. Remlinger and Riffat Bey<sup>4</sup> state that they have succeeded in passing the virus of

<sup>1</sup> *Zeitschr. f. Hygiene u. Infectiouskrankh.*, 1903, xliv. 507.

<sup>2</sup> *Journ. Exper. Med.*, 1913, xviii. 314.

<sup>3</sup> *Presse Médicale*, 1914, p. 79; also *Pathologica*, Jan. 1, 1914.

<sup>4</sup> *Compt. Rend. Soc. de Biol.*, 1903, lv. 730.



rabies through a Berkefeld filter ; if this be confirmed, it is necessary to conclude that the infective agent is capable of existing in a very minute form, at one period, at all events, of its life-cycle.

The virus or infective material, whatever its nature, resides in the saliva of infected animals, as is evident from the fact that the disease is most often conveyed by bites of rabid animals ; but it exists in still greater concentration in the central nervous system (brain and spinal cord). It is probably not present in the blood or in most of the organs of an animal which has died from the disease, but is found in the secretions of certain glands (lachrymal, mammary, pancreatic) ; possibly the poison is excreted by these channels. The toxin of rabies bears very close resemblance to that of tetanus in many of its properties. Thus it has a marked affinity for the nervous system, passing to the central portions of this by way of the peripheral nerves (*cf.* p. 139) ; it produces first a stimulation of the reflex activity of the nervous centres, though this is followed later on by paralysis ; its effects on the cerebrum are manifested by excitement and delirium analogous to the phenomena noted as the result of injections of tetanine into the brain-substance. Further, the occurrences in the wound itself bear some resemblance to those met with in tetanus, as in each case the injured point may cicatrize, but with the onset of the disease pain may occur in the scar ; while Pace<sup>1</sup> has shown that the virus of rabies remains locally at the seat of inoculation, as do the bacilli of tetanus. Deep wounds of a lacerated nature are those most liable to give rise to hydrophobia, just as injuries of this sort are those most commonly followed by tetanus.

Statistics as to the percentage number of all cases bitten by rabid animals which subsequently develop hydrophobia are somewhat difficult to obtain. Rose Bradford<sup>2</sup> puts it

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1903, xvii. 293.

<sup>2</sup> Art. "Hydrophobia" in "Quain's Dictionary of Medicine," 3rd edition, edited by Montague Murray. 1902.

at 16 to 25 per cent. ; some authorities give rather higher, others lower, figures. It is, at any rate, certain that all who are bitten do not develop the disease, even apart from treatment. This fact is of importance in estimating the benefits derived from preventive inoculations. When once it has appeared, the disease is invariably fatal. Persons bitten through their clothes are not very likely to be attacked by hydrophobia, as the virus is wiped off the teeth of the animal in passing through the dress-material. Natives of India and of other hot countries are thus more liable to suffer from hydrophobia than are Europeans resident in the same districts, owing to the scantiness of their clothing.

**Incubation period.**—The incubation period of hydrophobia is very long, varying from about three weeks to (possibly) some years. As to the extremely long periods assigned to the incubation of this disease, there is considerable doubt. Kaspareck and Teuner<sup>1</sup> relate a case in which the disease occurred seven months after infection, in spite of prophylactic inoculation. Pampoukis,<sup>2</sup> out of a number of cases not treated in any way, found that 9·3 per cent. occurred within the first month after the bite, 53·4 per cent. in the second month, and 37·2 in the third month. The average incubation period in the United States is said to be forty-nine days.<sup>3</sup> Probably six weeks may be looked on as the average period of time between the injury and the onset of symptoms.

**Modification of the virus of rabies.**—Although nothing is known of the poisonous material which gives rise to this malady, yet experiments show that it resides chiefly in the nervous system of infected animals, and that it can be modified in various ways. Thus, light, air, and desiccation rapidly destroy the virulence of rabic matter. Heat, also, has the same effect, and so has the addition of

<sup>1</sup> *Berlin. klin. Woch.*, 1902, Sept. 8, p. 844.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1900, xiv. 111.

<sup>3</sup> Leading article, *Med. Record*, 1912, lxxxi. 344.

antiseptic drugs, though the resistance offered to these last is considerable. Carbolic acid (1 : 20) cannot be relied upon to destroy the virulence of emulsions of brain-substance in less than an hour, and perchloride of mercury (1 : 1,000) takes three hours to sterilize this fluid. Digestion with gastric juice diminishes the virulence of infected spinal cords; and this method of producing a vaccine has been employed in Italy, and is known as the "Italian method." Post-mortem decomposition has little effect in destroying the virus of rabies, which may remain potent for at least a month after burial of a carcase. Glycerin is a good preservative of the virus (*cf.* Vaccinia).

Exaltation of virulence may be effected by passing the virus through a succession of rabbits, which are very sensitive to the disease. After passage through a large number of these animals the incubation period is gradually shortened from about three weeks or a little less to a constant period of six or seven days. Virus of this degree of virulence is called by Pasteur "*virus fixe*,"<sup>1</sup> and is used in the preparation of his vaccine. There is reason to believe that the virus which has thus been exalted in virulence for rabbits is really attenuated for mankind. Thus Nitsch<sup>2</sup> inoculated himself with fresh cord from a rabid rabbit, with no ill effects.

#### ANTIRABIC VACCINATION

**Pasteur's vaccine.**—Pasteur discovered that by drying the spinal cords derived from rabid animals for varying periods of time he could prepare a series of viruses of graduated strengths. Thus, if such a cord is dried for fourteen days, it loses all its toxic potency; if it is submitted to this process for only three or four days, the virulence is but little reduced. Immunity to rabies, as to other infective diseases, can be induced by injecting at first minute doses of the organism or toxin, and gradually increasing the

<sup>1</sup> As opposed to the virus of uncertain strength (*virus de la rue*; *Strassenwuth*) derived from accidentally infected animals.

<sup>2</sup> *Wien. klin. Woch.*, 1904, No. 36, p. 963.

doses until at last quite strong virus can be employed. Graduation of the dose is effected by taking equal amounts of nervous matter from spinal cords which have been dried for varying lengths of time. The actual vaccine consists of a small quantity (2-3 mm. length) of the substance of the spinal cord of a rabbit which has been killed by inoculation with the "fixed virus." This is rubbed up into an emulsion with 5 c.c. of sterile broth or salt-solution, and about 3 c.c. of the resulting fluid is injected. A cord dried for fourteen days is used for the first injection: on succeeding occasions emulsions of less attenuated virus are used, till finally a portion of a spinal cord dried for only three or four days is employed. A scheme of the actual doses may be thus drawn up:—

ORDINARY TREATMENT			INTENSIVE TREATMENT	
		Cord dried		Cord dried
First	day—Morning	14 days	2 injections	14 and 13 days
	Evening	13 "	"	12 and 11 "
Second	" Morning	12 "	"	10 and 9 "
	Evening	11 "	"	8 and 7 "
Third	" Morning	10 "	1 injection	6 days
	Evening	9 "	"	"
Fourth	" Morning	8 "	"	5 days
	Evening	7 "	"	"
Fifth	" Morning	6 "	"	"
	Evening	6 "	"	"
Sixth	" Morning	5 "	"	4 days
Seventh	" "	5 "	"	3 days
Eighth	" "	4 "	"	4 days
Ninth	" "	3 "	"	3 days
Tenth	" "	5 "	"	5 days
Eleventh	day "	5 "	"	"
Twelfth	" "	4 "	"	4 days
Thirteenth	" "	4 "	"	"
Fourteenth	" "	3 "	"	3 days
Fifteenth	" "	3 "	"	"
			On the following 6 days 6 more injections of 5-, 4-, 3-, 5-, 4-, 3-day cords respectively.	

A more rapid form of vaccination is used in cases in which the bites are about the face and head, as in these

cases the incubation period is usually shorter, and therefore it is important to produce a full degree of immunity as quickly as possible. This is known as "intensive" treatment. It will be seen in the scheme given that the virulent matter contained in a cord only dried for three days is here administered on the seventh day, instead of on the ninth, as in the ordinary method.

The exact arrangement of the doses varies a little at different institutions. Marx states that in Berlin it is considered that the virulence of the dried cord is lost about the eighth day, instead of the fourteenth. Hence the Berlin authorities consider that the first few days of the Paris treatment are wasted, only material which is quite inert being inoculated; they therefore adopt a scheme according to which, on the first day, cords dried for seven and eight days are administered; on the second day, cord of six days' drying; and so on, reaching a cord dried for three days on the sixth day of treatment. Then cords of five, four, and three days' drying respectively are each administered for two days, and on the fourteenth and fifteenth days cords dried for only two days. Then for the last four days of the treatment slightly less virulent material is again employed. In the intensive treatment at Berlin a cord of three days' drying is reached on the evening of the third day of treatment, and one of two days' on the eighth day. The whole intensive course, here also, lasts twenty-one days.

Nitsch<sup>1</sup> recommends starting with cord dried for six days, and giving two injections daily, reaching material that has been dried for only one day on the tenth (last) day of treatment. He uses also larger quantities of the vaccine (3-10 mm. of the cord) in emulsion.

Institutes for antirabic inoculation are now numerous. Besides the Paris "Pasteur Institute," there exist institutes at Lille, Marseilles, Montpellier, Lyons, and Bordeaux, in France; at Berlin, Vienna, Buda-Pest, Berne, Odessa, Constantinople, Algiers, Tunis, Kasauli (India), etc. Different

<sup>1</sup> *Wien. klin. Woch.*, 1904, No. 36, p. 959.

modes of preparing a virus of diminished virulence for purposes of inoculation are adopted in different countries. Thus, the Italian method of Tizzoni and Centanni is to treat the spinal cords with gastric juice, which has an attenuating effect on the virus. Hogyes, in Buda-Pest, merely dilutes an emulsion of virulent material to different degrees, using a high dilution for the first injections, and gradually raising the concentration on succeeding days. The theory underlying this procedure is that the usual method of attenuation by drying alters the quantity of the virus, but not its quality; in other words, it kills a certain proportion of the germs present, so that a smaller number of them are injected at a dose, but it does not alter their virulence. Hence, the same result may be obtained by simple dilution. The practical results of this method seem to bear out the theory on which it is founded, as very favourable statistics of the work of Hogyes' Institute are shown. Murillo<sup>1</sup> records the results obtained in 3,000 cases: 6 died after treatment and 17 before the course of treatment was completed.

**Effects of antirabic vaccination.**—The effect of Pasteur's method of vaccination in cases of bites by rabid animals is to produce an active immunity. Since the infective agent in rabies is not known, it is impossible to say with certainty whether the immunity depends on an antitoxin or on a germicidal state of the serum and tissues. The latter is the more probable, as it has already been shown that the virus must contain a living organism, not merely a toxin. Owing to the long incubation period of hydrophobia it is possible to induce immunity to the disease between the time at which the bite was inflicted and that at which the symptoms commence. Thus the treatment is in reality prophylactic, and not in any way curative. If the symptoms have already set in, Pasteur's treatment is of no avail. The analogy to ordinary vaccination (against small-pox) is exact. In the latter, vaccination carried out at the time of exposure to infection may protect against the disease,

<sup>1</sup> *Centralbl. f. Bakt.*, 1912, Orig., lxii, 606.

since the incubation period of vaccinia is shorter than that of small-pox. The difference here, however, is not very great, and more often such vaccination will only lessen the severity of the ensuing attack of small-pox. In the case of rabies, which has an incubation period of about six weeks as a rule, there is full time for immunity to be produced before the disease appears, and protection is thus usually complete.

**Results of the treatment.**—A good deal of scepticism was expressed as to the value of Pasteur's treatment when it was first introduced; it was even suggested that it might result in conveying the disease instead of preventing it, and

TABLE SHOWING DEATHS FROM HYDROPHOBIA AMONG CASES TREATED IN PARIS AND NEW YORK

Year	PARIS			NEW YORK		
	No. of cases	Deaths	Per-centage	No. of cases	Deaths	Per-centage
1886	2,671	25	0·94	—	—	—
1887	1,770	14	0·79	—	—	—
1888	1,622	9	0·55	—	—	—
1889	1,830	7	0·38	—	—	—
1890	1,540	5	0·32	160	0	0
1891	1,559	4	0·25	100	2	2
1892	1,790	4	0·22	104	0	0
1893	1,648	6	0·36	85	0	0
1894	1,387	7	0·50	89	1	1·12
1895	1,520	5	0·33	167	2	1·19
1896	1,308	4	0·30	236	0	0
1897	1,521	6	0·39	133	1	0·74
1898	1,465	3	0·20	125	1	0·8
1899	1,614	4	0·25	159	2	1·2
1900	1,420	4	0·28	241	1	0·43
1901	1,321	5	0·38			
1902	1,105	2	0·18	—	—	—
1903	628	2	0·32	—	—	—
1904	755	3	0·39	—	—	—
1905	727	3	0·41	—	—	—
1906	772	1	0·13	—	—	—
1907	786	3	0·38	—	—	—
1908	524	1	0·19	—	—	—
1909	467	1	0·21	—	—	—
1910	401	0	0·00	—	—	—
1911	341	1	0·29	—	—	—
1912	395	0	0·00	—	—	—



it is possible that accidents of this kind have actually occurred. At the present time there can no longer be any doubt as to its efficacy, or as to the boon conferred on the human race by its discovery. The exact mortality from hydrophobia in all cases of bites by rabid animals, in times before the inoculation treatment was introduced, cannot be exactly calculated, but it may safely be put at not less than 10 per cent., whereas now among the cases treated at the various Pasteur Institutes the death-rate has been reduced to a fraction of 1 per cent. The table on p. 175 shows the annual mortality at two separate institutions—the original Pasteur Institute in Paris<sup>1</sup> and the similar foundation in New York.<sup>2</sup> It will be seen from these figures that the death-rate has never reached 1 per cent. in Paris since the Institute was started, while in New York the percentage was only thrice over that amount in the years recorded above.

Very full statistics are published by the various Pasteur institutions as to the exact nature of the cases treated, in which these are tabulated according to the region of the bite and the evidence available as to the reality of the disease from which the dog or other animal that inflicted the injury was suffering. In the tables on pp. 177–8, Class A contains cases in which the dog was proved by conclusive evidence to be rabid; Class B, those in which rabies was certified by a veterinary surgeon, as a result of examination; and Class C, those in which the nature of the disease in the animal was doubtful. The injuries are classified, as a rule, according as they were on the face, hands, or lower limbs, the last being usually covered with clothes.

In the table on p. 178 are given the figures supplied by the Indian Pasteur Institute at Kasauli,<sup>3</sup> under Major D.

<sup>1</sup> Viala, *Ann. de l'Inst. Pasteur*, 1913, xxvii. 794.

<sup>2</sup> Rambaud, *Med. News*, 1902, i. 635. We have not been able to find the New York statistics for later years.

<sup>3</sup> *Annual Report of the Sanitary Commissioner with the Government of India*, 1901, p. 128.

Simple, M.D., R.A.M.C., which are on a slightly different system, the classes being, however, the same. The statistics for Europeans and natives are given separately, the latter being liable to more extensive and dangerous bites owing to their lighter clothing. In 1905, 1,145 persons were treated, with 7 failures; and in 1906, 1,147 persons, with 9 failures.<sup>1</sup>

TABLE OF CASES TREATED IN THE PASTEUR INSTITUTES OF PARIS (1901) AND NEW YORK (1900-1)

	Bitten on head			Bitten on hands			Bitten on lower limbs			Total		
	Treated	Died	Mortality	Treated	Died	Mortality	Treated	Died	Mortality	Treated	Died	Mortality
Class A	20 13	0 1	0 7·69	93 62	0 0	0 0	58 13	0 0	0 0	171 88	0 1	0 1·13
Class B	80 7	0 0	0 0	521 47	4 0	0·77 0	184 6	0 0	0 0	785 60	4 0	0·51 0
Class C	23 13	1 0	4·34 0	186 53	0 0	0 0	153 27	0 0	0 0	362 93	1 0	0·23 0
Total	123 33	1 1	0·79 3·03	800 162	4 0	0·50 0	395 46	0 0	0 0	1318 241	5 1	0·38 0·4

In the above table the figures derived from the New York Institute are in dark type.

Ferré<sup>2</sup> records that at Bordeaux there were treated, in 1901, 100 cases of bites by rabid animals, with no deaths. Trolard,<sup>3</sup> in Algiers, treated 1,836 patients, among whom there occurred 9 deaths (0·49 per cent.). In Tunis,<sup>4</sup> up to the end of 1906, 2,490 cases had been treated, with 9 deaths—a mortality of 0·36 per cent.

<sup>1</sup> *Annual Report of the Sanitary Commissioner with the Government of India*, 1905 and 1906.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1902, xvi. 391.

<sup>3</sup> *Ibid.*, 1900, xiv. 190.

<sup>4</sup> Nicolle, *Arch. de l'Inst. Pasteur de Tunis*, 1907, i. 35.

TABLE SHOWING RESULTS OBTAINED IN THE INDIAN PASTEUR INSTITUTE, 1901

CLASSES	SUB-CLASS I BITTEN ON THE HEAD OR FACE			SUB-CLASS II BITTEN THROUGH THE EX- POSED SKIN ON ANY PART OF THE BODY OTHER THAN THE HEAD OR FACE			SUB-CLASS III BITTEN THROUGH THE CLOTHING			TOTALS		
	Treated	Failures	Per- centage mortality	Treated	Failures	Per- centage mortality	Treated	Failures	Per- centage mortality	Treated	Failures	Per- centage mortality
CLASS A— Bitten by animals { proved rabid	9	0	0	63	0	0	14	0	0	86	0	0
	6	0	0	81	3	3.7	5	0	0	92	3	3.26
CLASS B— Bitten by animals { certified rabid	2	0	0	43	0	0	7	0	0	52	0	0
	1	0	0	38	0	0	3	0	0	42	0	0
CLASS C— Bitten by animals { suspected rabid	7	0	0	47	0	0	23	0	0	77	0	0
	15	0	0	155	2	1.29	24	0	0	194	2	1.03
TOTAL ... ..	18	0	0	153	0	0	44	0	0	215	0	0
	22	0	0	274	5	1.83	32	0	0	328	5	1.52

In the above table the figures relating to Europeans are in dark type.

It should be noted that although we have exemplified certain points by figures from the first year of this present century (*see* tables on pp. 177-8), the results of treatment are so good that statistics from any year since 1888 would have afforded equally convincing evidence.

**Site of injection.**—The immunizing injections are generally administered subcutaneously over the abdomen, as here it is easy to avoid injury to any nerves. Krasnitski<sup>1</sup> recommends intravenous injection of a filtered emulsion, as producing a more rapid protection. He states that he has successfully treated 70 cases in this manner without any ill effects.

The importance of *early treatment* after the injury has been inflicted is proved by the statistics of the Odessa Institute,<sup>2</sup> which show that of 4,602 cases treated within the first week, 26 deaths occurred, giving a mortality of 0·56 per cent. ; among 961 treated in the second week, 16 died, or 1·66 per cent. ; while among 313 treated in the third week, 10 deaths ensued, a mortality of 3·19 per cent.

## SERUM TREATMENT

**Antirabic serum.**—The serum of animals immunized by the Pasteurian method is capable of neutralizing the virus of the disease. If a sufficient amount of the serum be mixed with an emulsion of virulent spinal cord and injected into a rabbit, no symptoms of disease will develop. As previously mentioned, in the absence of all knowledge of the causal agent of hydrophobia it is impossible to ascertain whether the serum is antitoxic or germicidal ; but probabilities are in favour of the latter. Tizzoni and Centanni,<sup>3</sup> as the result of prolonged experiments, suggested the use of this serum as a protective against the disease in persons who had been bitten, instead of the Pasteurian treatment.

<sup>1</sup> *Arch. de l'Inst. Pasteur de Tunis*, 1907, i. 393.

<sup>2</sup> Quoted by Deutsch and Feistmantel, "Impstoffe und Sera." Leipzig, 1903.

<sup>3</sup> *Lancet*, 1895, ii. 659, 727, and 780.

They consider that their method is quicker and equally certain. In some cases, also, this serum may act as a cure (in rabbits) when the symptoms of the disease are just beginning, a period at which ordinary immunizing treatment would be absolutely useless.

**Preparation.**—The method of preparing the serum is by inoculating sheep with rabic material attenuated by the "Italian method" (p. 171). For the first series of inoculations, 17 injections in all are given over a period of twenty days, each dose consisting of 0.25 grm. of virus for every kilogramme of body-weight. The injections are given subcutaneously. Later on, immunity is kept up by further inoculations at intervals of two or two and a half months. The serum is withdrawn on the twenty-fifth day after the last injection. The fresh serum may be dried at a gentle heat over sulphuric acid, and preserved in this form indefinitely.

The serum thus prepared will protect animals against rabies when administered in doses equivalent to  $\frac{1}{25000}$  of the body weight. One and a half drops may protect an animal weighing 2 kg. A serum of this strength is called 'typical serum' (S.T.).

Tizzoni and Centanni state that their serum is applicable to man, and recommend that doses of 20 c.c. should be used, given in three injections—one half first, then the remaining half in two other doses at intervals of three days. The above amount is advised for cases which come under treatment within the first four days after the bite. For cases seen between the fourth and fifteenth days the amount of serum should be doubled, and very large quantities should be given in cases of bites about the face and head.

Serum treatment has been carried out at the Kasauli Institute by Lieut.-Colonel Semple in the case of badly bitten persons and of those who come for treatment some time after being bitten. The serum is prepared from ponies. "A dose of serum is given on the first day, after which the usual methods of treatment are carried out." It is hoped

that a passive immunity may thus be induced, so as to render the patient safe against infection during the period before the active immunity comes into play.<sup>1</sup> It would be well to inject some of the serum in any case in which pain or discomfort began to be felt in a wound inflicted by a bite, after this had healed up, even before any symptoms of hydrophobia were manifested. The serum is quite harmless in any case.

Tonin<sup>2</sup> records a case of hydrophobia cured by the injection of *salvarsan*, although Marras<sup>3</sup> found that the drug was valueless in the treatment of rabies experimentally produced in the laboratory.

### CONCLUSIONS

1. If possible, in cases where there is doubt as to whether a dog which has bitten anyone is rabid or not, the animal should *not* be killed at once, but should be kept under close observation. In this way a positive diagnosis can usually be made in less than ten days; otherwise it may be necessary to have recourse to experimental inoculations to decide the question, and such experiments take three to four weeks. It would not be safe to await the results of these experiments before beginning treatment.

2. In all cases of bites by rabid animals, recourse should be had as soon as possible to antirabic inoculation. It is important that this should not be delayed. There is practically no danger in the procedure.

3. If for any reason the preventive treatment has been put off till unduly late, it would seem advisable to inject antirabic serum as a prophylactic measure, should it be available.

<sup>1</sup> See *Ann. Report of the Sanit. Commissioner with the Govt. of India*, 1902, p. 115; Semple, *Lancet*, 1908, i. 1611.

<sup>2</sup> *Il Policlin.*, Sez. Pract., 1912.

<sup>3</sup> *Centralbl. f. Bakt.*, I., Orig., 1912, lxx. 190.

## CHAPTER IX

### SMALL-POX AND VACCINIA

**Causal agent.**—That small-pox is due to some living agent similar in nature to the organisms of other infective diseases there can be no doubt, but the actual germ has not been certainly isolated. The most probable parasite is that named by Guarnieri the *Cytoryctes variolæ*, which may be identical with that described by Councilman and by Calkins. The parasites described by the latter authors are said to pursue a double life-cycle within the cells, one phase being extranuclear, the other intranuclear. The latter is supposed to correspond with the sexual cycle of the malarial parasite. It is suggested that vaccinia represents the extranuclear phase of the organism, whereas small-pox consists essentially in the invasion and destruction of the nuclei. If these observations are confirmed, the discovery will be a matter of great interest. We shall have an instance of the attenuation of a protozoan parasite taking place by passage through another animal, just as occurs in the case of vegetable parasites (bacteria). Further, a vaccine will have been prepared against a protozoön<sup>1</sup> as well as against bacteria, showing that the human body has the power of forming protective substances against this order of pathogenic agents, as well as against vegetable organisms. Again, the suggestion of an organism undergoing two different cycles within the same animal host, but in different positions (cell and nucleus), is of considerable interest.

<sup>1</sup> Ledoux-Lehard (*Compt. Rend. de l'Académie des Sciences*, 1902, cxxxv. 298) states that he has prepared a specific antiserum to the protozoan organism, *Paramœcium caudatum*, which is pathogenic to some of the lower animals (rabbits and guineapigs).



**Identity of small-pox and cow-pox.**—The question of the identity or difference of small-pox and cow-pox was long disputed, but there can now be little doubt that Jenner was right in holding that the latter is only small-pox modified by transmission to a different animal which is less susceptible to the disease.<sup>1</sup> Many attempts have been made to transmit small-pox directly to cattle, and a certain number of successful results have now been recorded. Adult cows take the disease with difficulty; calves are more easily infected. A condition in horses analogous to vaccinia (equine variola) appears to be really the same disease, and lymph from this source is capable of effecting vaccination.

**Complications of small-pox.**—Most of the complications arising in small-pox can be distinctly traced to intercurrent infection with pyogenic micrococci (pneumococci, streptococci, or staphylococci). It has even been said that the pustular stage of the lesions, which has been regarded as so characteristic of small-pox, as opposed to chicken-pox, can be almost entirely prevented by careful and thorough antiseptic treatment of the skin. If this be true, it would seem that the cutaneous manifestation of the disease is essentially a vesicular eruption, and only accidentally becomes pustular. Abscesses are the commonest complication met with; erysipelas and cellulitis are by no means rare. The ocular affections (keratitis and conjunctivitis) may possibly be due to the virus of the original disease, but here again the action of secondary infections can hardly be excluded. Pneumonia, pleurisy, and empyema are also probably instances of intercurrent infection.

#### VACCINATION

**Theory of vaccination.**—As already explained, vaccination consists in inoculation of an attenuated form of small-pox germs, the diminution in virulence being brought about by passage through the body of a calf, a less suscep-

<sup>1</sup> See Blaxall: *Thirty-first Ann. Rept. of the Med. Off. of the Local Govt. Board*, 1901-2. Appendix C, i. 568.

tible animal than man. The attenuated germs are present in the lymph of the vesicles formed on the vaccinated person, and this lymph may be used for inoculation of other individuals, as the germs do not regain their virulence by repassage through man. Vaccinia remains a localized disease, the attenuated germs remaining in the place of inoculation, and not becoming generalized by the blood-stream. At the point of inoculation they form their toxins, which are conveyed all over the body, and stimulate the tissues to form germicidal substances. The cells thus educated retain the property of secreting these substances for a considerable length of time ; in other words, the person vaccinated has gained an active immunity to small-pox and vaccinia.

**Preparation of lymph.**—It is immaterial, theoretically, from what source, human or bovine, the lymph is derived, but, for reasons set forth below, the use of material from an “animal” source is to be preferred in practice. What is known as “glycerinated calf-lymph” is now chiefly used in this country.<sup>1</sup> This is prepared in the following manner: A supply of stock lymph being already available, a calf is taken, and its abdomen is shaved. A series of parallel incisions are made in it of considerable length, and the stock lymph is rubbed into them. By the fifth day large vesicles have developed along the course of the incisions, and are full of clear fluid which does not yet exhibit any tendency to become pustular. At this stage the vesicles and their contents are scraped off with a sterile sharp spoon, with all aseptic precautions, and the resulting material is collected in suitable bottles. It is next finely broken up, and triturated with four times its weight of glycerin and water (50-per-cent. solution). The thick creamy fluid produced is run into tubes, and these are kept in a cold, dark place for some weeks. The result of this treatment is to kill off most of the common pyogenic and similar organisms which might do harm if inoculated; even so but few

<sup>1</sup> In India, in addition to glycerinated lymph, mixtures with vaseline and with lanoline are also employed, apparently with good results.

specimens of lymph are actually free from extraneous bacteria. The contagium of vaccinia is left apparently uninjured. It is possible that it exists at this stage in the form of spores, which are resistant to the action of the glycerin. After about a month the lymph is examined bacteriologically, to ascertain whether it is free from gross bacterial contamination; and if it is found to be innocuous, it is drawn into capillary tubes and is ready for use. The lymph thus prepared is a thick, syrupy fluid, which tends to separate to some extent into a clear and an opaque portion. It is probable that the latter is the active part, and care should therefore be taken not to use only the clear portion in vaccinating.

“Chloroform” calf-lymph, first introduced by Green,<sup>1</sup> is also extensively used in England at the present time. This is prepared as follows: The lymph is first mixed with water for the purpose of this procedure. Air charged with the vapour of chloroform is then made to pass through a series of tubes of vaccine, and the chloroform is subsequently expelled from the tubes by means of a current of air; thus only the proportion of chloroform which the water can hold in solution (1:400) can come in contact with the vaccine. This quantity suffices to kill the bacteria (chiefly staphylococci) generally present, but has no ill effect on the vaccine virus. The addition to this of any traces of liquid chloroform appears, however, to diminish its activity. The advantages claimed for this method are the efficiency and the speed with which the destruction of bacteria is effected, so that in cases of emergency large quantities of vaccine can be rapidly rendered fit for use, and the consequent avoidance of any possible deterioration of strength, such as may perhaps occur during the month or more for which ordinary glycerinated lymph has to stand.

**Technique of vaccination.**—The essential point in the process of vaccination is that the infective material—the lymph—should be introduced through the epidermis, so

<sup>1</sup> *Lancet*, 1903, i, 1738.

as to be absorbed by the lymphatics and blood-vessels of the corium. The operation itself is carried out in the following manner: The *site* usually chosen is the skin of the outer side of the upper arm over the insertion of the tendon of the deltoid muscle; sometimes, in females, on the outer side of the thigh just below the great trochanter, or well above the knee on the inner or outer aspect of the thigh. The skin at the seat of operation is, if hairy, carefully shaved, and, in any case, well washed with soap and water and then thoroughly scrubbed with ether. After the ether has evaporated, a tube of lymph is opened, and the whole of its contents ejected on to the clean skin by means of a rubber bulb provided with a plug of sterilized cotton-wool; a sterile lancet or scarifying needle is used to scratch the epidermis *through* the lymph, care being exercised to avoid drawing blood: the lymph is then rubbed into the scratches. The superfluous lymph is collected on the blade of the lancet and transferred to another spot on the skin about an inch distant, and the process is repeated. Three or four "insertions" are usually made, to obviate risk of failure. The several sites of inoculation should not be too close together—preferably an inch or an inch and a half should be left between them—in order that the resulting vesicles may not coalesce. Should they do so, an unduly sore arm may ensue, and a considerable amount of scarring be finally left. The lymph is left for a few minutes to soak in, and the remainder is wiped off the skin with sterile cotton-wool. The wounds are then dressed with dry sterile gauze, bandaged on, or a thin layer of collodium flexile is painted over the scarifications.

Nobl<sup>1</sup> has made trial of *subcutaneous injection* of vaccine-lymph in doses of 0.1 to 0.2 c.c., diluted with normal saline solution. He finds that there is less local reaction, and no greater constitutional disturbance than with the ordinary method. Immunity is rather later in appearing (tenth day). Nobl believes that the method will prove useful in that it

<sup>1</sup> *Wien. klin. Woch.*, Aug. 9, 1906.

avoids risk of local infection and of auto-inoculation ; the dose, too, can be exactly measured, and no scars result.

**Phenomena of vaccination.**—When properly performed, with that careful attention to detail that should characterize every bacteriological procedure, whether carried out in the laboratory or on the operating-table of the surgeon, vaccination results, in from five to eight days, in the formation of a small vesicle with a sharply raised edge, filled with clear serum, and surrounded by a narrow red areola. During the next few days the centre of the vesicle sinks so as to form a cup-shaped depression, and the fluid contents of the surrounding portion of the vesicle become white and opaque. By the tenth to the twelfth day the vesicle has become a dry scab, which finally separates and leaves a circular depressed scar—"foveated."

About the third day marked constitutional disturbance is noted—rise of temperature, headache and general malaise, and occasionally sickness and diarrhœa, usually accompanied by some itching at the seat of inoculation. These symptoms are generally present until the vesicle is well developed (eight to ten days), after which they gradually subside. The neighbouring lymphatic glands are enlarged and tender, from about the fifth to the tenth or twelfth day. The "beautiful arm" so commonly seen after vaccination in the past century, with its vesicles rapidly becoming pustular, and the accompanying extensive and severe cellulitis, often reaching to the wrist and hand, was no criterion of efficient vaccination: it merely formed a pointed commentary upon the contaminated lymph and septic methods of that age.

**Revaccination.**—In those who have already been once or more vaccinated the phenomena are similar, but less marked. Only a papule may appear, or a poorly developed vesicle with subsequent scabbing. Itching may be the most marked feature. Not very infrequently in such persons revaccination fails entirely.

**Number of insertions.**—Statistics prove that the protection afforded by primary vaccination is to some extent

proportional to the number of spots at which the lymph is inserted, two "scars" protecting better than one, three than two, and four than three. The practice of making only one insertion is to be condemned as inefficient and conveying a false security. The scars resulting from the vaccination should together make up an area of not less than half a square inch. The following table gives an example of the statistical evidence upon which these statements are founded.

TABLE SHOWING THE AGE-INCIDENCE IN VACCINATED CASES CLASSIFIED ACCORDING TO THE CHARACTER OF THE SCARS; EACH CLASS IS REPRESENTED AS COMPRISING A TOTAL OF 1,000 CASES (SANDILANDS)<sup>1</sup>

Character of scar	Under 10 years	10-	20-	30-	40-	50 and upwards
Large (A 1) ... ..	15	187	411	253	98	36
Medium (A 2) ... ..	22	109	248	268	222	131
Small (A 3) ... ..	45	112	199	241	211	192
Four or more ... ..	23	217	441	207	77	35
Three ... ..	11	148	350	309	127	55
Two ... ..	15	114	273	292	190	116
One ... ..	31	129	270	236	203	131
Half or more than half foveated ... }	21	188	418	246	99	28
Less than half foveated ... }	15	183	387	263	107	45
Plain scars ... ..	27	127	293	238	182	133
Scars absent... ..	83	204	210	157	127	219

Dr. Sandilands points out that "the figures in this table demonstrate a point of some importance—that the incidence in later life is very much greater in the classes with inferior vaccination scars." This point is well brought out in the last column, where it will be seen that those cases exhibiting the depressed scar typical of a successful vaccination have a case-incidence of less than 3 per cent., whilst among those showing the plain scar, resulting from infection with pyogenic organisms only, the incidence is five times as great.

<sup>1</sup> "An Analysis of the Vaccination Statistics of the Metropolitan Asylum Board for 1901 and 1902," *Lancet*, 1903, ii. 378.



It seems at first sight rather difficult to understand the reason for the relation borne by the amount of protection to the area of vesicles resulting, since it might be supposed that, vaccinia being an infective disease, the virus would multiply in the body in any case to an extent only limited by the resistance of the individual, and that therefore one insertion would be as effective in producing immunity as many. The facts being as stated, it seems that the infective organism, whatever its nature, remains localized, in the majority of instances at any rate, within the tissues near the site of inoculation, multiplying to some extent therein, and producing poisons which are carried throughout the system. It would thus bear a close resemblance to the bacilli of diphtheria and tetanus in its mode of behaviour. It is requisite that a certain amount of the poison should be manufactured, in order to cause a sufficient action on the cells of the body to stimulate the formation of the necessary amount of protective substances. Hence the need for a considerable quantity of the virus to be inoculated, the size of the dose being gauged by the number of scars, since possibly the organisms tend to die out somewhat rapidly, being *ex hypothesi* of an attenuated kind. It seems not impossible that a fallacy of observation may lurk in the inference drawn from the statistics, since it must be recollected that the relationship of scar-area to immunity was predicated in the days of septic lymph and haphazard technique. It may well be that one aseptic successful insertion will ultimately prove to be as protective as the four hitherto insisted upon as the counsel of perfection; and that it is not so much the number of insertions of the lymph that protects, as the careful performance of the act of vaccination, of which the number of scars is some criterion.

It is well to remember that the lymph remaining on the arm or other part vaccinated may be *conveyed accidentally* to other regions of the body, and that if there is any excoriation at the point of contact a vaccination lesion will result.



Should such an occurrence take place on the face, a somewhat alarming condition is often produced, the affected part swelling markedly and the neighbouring glands enlarging to a considerable size.<sup>1</sup> The condition is in no way dangerous, but an unsightly scar may be left. A generalized eruption is sometimes produced by such accidental inoculation if it occur in several places.

**Risks of vaccination.**—In the days when it was the practice to vaccinate one child from another by the “arm-to-arm” method, there was a certain element of risk lest some disease should be transferred from one to the other at the same time. Thus it can hardly be denied that *syphilis* has been conveyed in this manner; it seems definitely established that the clear lymph of a vesicle may convey the infection, even apart from contamination with blood, and the possibility of the transference becomes obvious since the demonstration of spirochaetes in the congenital syphilitic. This risk no longer exists when calf-lymph is used.

Secondary infection may take place at the site of a vaccination puncture, as it may by any other abrasion of the skin. Thus, in a certain number of cases, *erysipelas* has supervened, owing to the subsequent entry of streptococci derived from the insanitary surroundings of the child. It is said that the vaccination in such cases is generally unsuccessful. Milder *septic infection* (probably with staphylococci) may result in a sore arm of unusual severity, and even give rise to glandular abscesses.

In countries where *tetanus* is common, this complication has followed vaccination. MacFarland,<sup>2</sup> from a study of 95 such cases, concludes that, although such infection has most often been due to subsequent contamination of the vaccinated area, in some cases the actual lymph may have

<sup>1</sup> The picture thus presented has on more than one occasion been regarded as due to infection by *B. anthracis*, and heroic measures of treatment, such as excision, have been adopted.

<sup>2</sup> *Journ. of Med. Research*, May, 1902.

contained tetanus spores derived from hay, manure, etc. Scott<sup>1</sup> records two cases of tetanus among 30,000 persons vaccinated in St. Louis.

The constitutional disturbance produced by vaccination may in some cases be prolonged, taking the form of somewhat severe *anæmia*. Bellotti,<sup>2</sup> who calls attention to this possible sequel, states that children who have been previously rosy and healthy in appearance most often exhibit this condition. He suggests that the organisms of vaccinia may in these rare cases exert a special hæmolytic action.

The names *vaccinia hæmorrhagica* and *vaccinia gangrenosa* have been applied to conditions in which symptoms of unusual severity attend vaccination. In the former a generalized hæmorrhagic eruption develops, which may be accompanied by bleeding from mucous surfaces; in the latter the local lesions, instead of healing, extend deeply and widely, causing necrosis of the tissues and large areas of ulceration, together with severe constitutional disturbance.

It is probable that these conditions are both dependent in the first place upon a debilitated condition of the child, produced by ill feeding, rickets, or tuberculosis; and in the second place upon an invasion by other organisms, such as pyogenic cocci, which either produce local gangrene in the weakened tissues, or give rise to a general septicæmic condition, with hæmorrhagic symptoms. Of the close connection between hæmorrhagic eruptions and general septicæmic states there can be no doubt whatever.

In a third extremely rare condition, known as *generalized vaccinia*, successive crops of vesicles develop upon various parts of the body and upon the mucous membranes of the mouth, nose, eye, and genitals, at short intervals, until in severe cases only a few small areas of normal skin remain. It may be due on the one hand to extreme

<sup>1</sup> *Medical Record*, 1910, lxxviii. 811.

<sup>2</sup> *Gaz. degli Ospedali*, May 10, 1903.

virulence of the germs inoculated, or on the other to extreme susceptibility of the vaccinated individual.

A *cheloid* condition sometimes results from the scarring produced in vaccination. This probably has nothing to do with the virus employed, but depends upon a constitutional peculiarity of the individual, in whom any slight traumatism may give rise to a chronic inflammatory over-production of scar-tissue.

In the absence of an epidemic of small-pox a child should not be vaccinated when it is obviously in bad health. Not only will the parents attribute to the operation any increase in the symptoms of the existing condition which may ensue, however accidentally—so that the procedure will incur some degree of disrepute with them and with their ignorant neighbours, which it is better to avoid—but it is probable that in some instances the constitutional disturbance produced by the inoculation may unduly depress a child already weakened by existing disease. Children suffering from eczema, herpes, or other skin-disease should not be vaccinated, if the matter is not urgent. Generalization of the vaccinal eruption is said to occur in such patients, but the evidence is not very clear. Hæmophilic subjects should either not be vaccinated, the risk to them being greater from any source of bleeding than from the continued liability to small-pox; or they should have the lymph introduced by subcutaneous injection.

**Insusceptibility to vaccination.**—It is said that some persons are by nature insusceptible to vaccination. This may possibly be the case occasionally, but instances of such a condition which will stand investigation must be very rare indeed. Thus, Thorne<sup>1</sup> states that 107,180 vaccinations have been done by public vaccinators under the Local Government Board without one instance of failure. Cory<sup>2</sup> reports one case, among 38,000, in which

<sup>1</sup> *Twenty-seventh Ann. Rept. of the Med. Off. of the Local Govt. Board*, p. viii.

<sup>2</sup> "Lectures on Vaccination," p. 73.

he was twice unsuccessful in attempting to vaccinate an infant. Bryce<sup>1</sup> records 98 unsuccessful attempts to vaccinate with calf-lymph out of 126,000 vaccinations. According to English law, it is necessary to make three attempts at (primary) vaccination before pronouncing any individual insusceptible. It is not unusual, in attempting to revaccinate an adult, to find it impossible to produce any effect recognizable as vaccinia. The same is of course true, and to a still greater degree, of those who have suffered from small-pox.

**Supply of lymph.**—In the present state of the law in this country, public vaccinators are supplied by the Local Government Board with lymph which is prepared under careful State supervision. This lymph is not to be obtained by other practitioners, who are dependent for their material upon the lymph offered in the market by private trading establishments. No supervision of any kind is exercised over these manufactories, so that only the pressure of competition with other firms, and the risk of losing custom if their product is found inert, are to be relied upon to ensure the purity and efficacy of these lymphs. Such a state of things appears entirely indefensible. It is much to be hoped that, in future Acts of Parliament dealing with vaccination, provision will be made for the inspection of private vaccine establishments, and for the testing by State officials of all lymph put upon the market, with regard to its activity, and also to its freedom from bacterial contamination. Although eleven years have elapsed since the issue of the first edition of this book, no step has yet been taken in this direction.

**Protection afforded by vaccination.**—Of the value of the protection afforded by vaccination against small-pox there can be no doubt in the mind of anyone who is willing to look facts in the face and draw conclusions without pre-existing bias. Before Jenner introduced his great discovery to the world the disease was universally prevalent.

<sup>1</sup> *Boston Med. and Surg. Journ.*, Feb. 26, 1903.

It was regarded as a children's disease, owing to the fact that all were susceptible and contracted small-pox at the earliest opportunity. It thus caused an immense infantile mortality; but it also attacked adults of all ages and all positions in life. Princes were no more sacred from its attack than the poor; scarred faces were the rule rather than the exception. Hence the new prophylactic was welcomed with delight throughout the world, and special measures were taken to introduce it and to carry a supply of lymph into the most distant countries.

At the present day, owing to the general practice of vaccination, small-pox is a rare disease, and its very rarity has caused a certain degree of carelessness in carrying out the prophylactic procedure. Hence there are signs that in this country the disease is making attempts to re-assert itself; and places such as Gloucester and Leicester, where the fanatical opponents of vaccination have gained the ascendancy and succeeded in causing general neglect of the precaution, have paid the penalty for their folly by suffering from severe epidemics.

The general recognition of the value of vaccination is shown in the regulations adopted by most life-insurance offices, which charge an additional premium to all those who have not been vaccinated. In view of the general protection of the community, the risk is small and the addition slight; but there can be no doubt that, if small-pox once more became prevalent, this additional percentage would be considerably increased. Vaccination, or revaccination, is also compulsory upon recruits for the army and navy, and upon those employed in the postal service.

The statistics of the German army and of the civil population<sup>1</sup> in that country afford convincing evidence of the benefits derived from vaccination, if any be still needed. When compulsory vaccination was introduced into the army the average deaths per 100,000 (taking an average

<sup>1</sup> See Statistical Chart quoted in Marx, "Die Experimentale Diagnostik, Serumtherapie u. Prophylaxe der Infektionskrankh.," 1907.

of the 10 years before and the 10 years after its initiation) fell from 36 to 3, whereas in the civil population, not thus protected, the relative numbers remained 26.9 and 19.4 respectively, showing no such tendency to fall. At the same time the existence of small-pox among the civil population was a source of infection even to the protected members of the army, a small number of cases continuing to occur. It was only after vaccination was enforced universally throughout Germany that the disease practically disappeared from the army, while among the civil population also it at once fell almost to vanishing-point.

We may prove the value of the protection thus afforded by vaccination, by means of a comparison of the German army with others not so protected. In the German army, from 1875 to 1887, only 148 cases of small-pox occurred, whereas in the Austrian army, not protected by systematic vaccination, there were 10,238 cases between 1873 and 1886, and in the French army, from 1875 to 1881, 5,605 attacks.<sup>1</sup> In Sweden, in prevaccination days, 2,050 deaths occurred annually from variola; after vaccination was introduced the average mortality fell to 169 per annum. In Bohemia, with a population of 3,039,722, the average annual deaths from small-pox were 7,663; after vaccination was introduced they fell to 282, though the population had risen meanwhile to 4,248,155. Thus the small-pox mortality fell, owing to vaccination, from 1 in 397 of the population to 1 in 14,741—a sufficiently striking decrease.

There is no doubt that Jenner was wrong in considering that vaccination once performed conferred an absolute immunity against small-pox; and failure to recognize certain limitations in this respect has done harm, by enabling disbelievers in the practice to create a distrust in the minds of the ignorant by pointing to individual instances of failure, where complete protection

<sup>1</sup> Immermann, art. "Vaccination" in Nothnagel's "Encycl. of Pract. Med.," English ed., 1902.

had been promised. That a person who has been once vaccinated may afterwards suffer from small-pox is undoubted, although it is almost always the case that a subsequent attack of the disease, if it should occur, is relatively mild (modified small-pox). A certain number of deaths do, however, occur even among those who have been vaccinated. Even revaccination does not necessarily confer absolute immunity.

The explanation of this is not difficult. In the first place, there is now no doubt that in many persons the period of immunity after vaccination is not indefinitely prolonged. Perhaps seven years may be taken as the average period of fairly complete protection, but probably even during this time the degree of resistance is constantly diminishing. In the second place, it is most probable that modifications of general health may affect the individual's resistance to this, as to other diseases, even when immunity has been produced. Fatigue or ill-health may perhaps temporarily reduce the powers of defence. The longer, therefore, the period which has elapsed after vaccination, the less the degree of protection that is likely to persist, and the more easily will depressing circumstances suffice to reduce it below the point necessary to confer immunity. The following table shows the gradually diminishing protection afforded by vaccination, and the consequent increase of mortality as age advances :—

TABLE SHOWING THE PERCENTAGE MORTALITY AT SEVERAL AGE-PERIODS AMONG THE SAME SCAR-BEARING VACCINATED CASES AS ARE SHOWN IN THE FORMER TABLE, p. 188 (SANDILAND)<sup>1</sup>

	Under 10 years	10-	20-	30-	40-	50-	60-	70 and upwards
Mortality      ...      ...	3	2	7	5	22	21	24	20

<sup>1</sup> *Op. supra cit.*



Hence it cannot be too strongly insisted upon that not only vaccination, but *revaccination*, is needful to protect the individual and society against small-pox. Children should not only be vaccinated soon after birth—within the first three months of life—but revaccinated, perhaps on going to school, and certainly on leaving it. Should small-pox be at all prevalent, adults will be wise to have the operation repeated if more than seven years have elapsed since they last underwent it. If as a matter of fact they are still immune, the vaccination will not “take,” and they will suffer no inconvenience; while if it succeed, they will have the satisfaction of knowing that they have gained a new lease of immunity.

In this connection we may quote the following remarks by Dr. Sandiland<sup>1</sup> with regard to the protection of the community at large by vaccination:—

“It cannot be too much emphasized that the extraordinary diminution in the mortality from small-pox in the last century has been due, not so much to the protection of a majority of the population, as to the absolute immunity of a minority, probably made up from persons at all periods of life, who are continually standing in the way of small-pox infection, and compelling it to travel by long and circuitous routes before alighting, scattered and diluted, on patches of soil in which it can take root and flourish.

“Again, a person saved from small-pox by vaccination should not, so to speak, be counted as one, but rather should be represented by a figure standing for himself and all those whom he would have infected had he been overtaken by the disease. It is this process of the multiplication of the benefits of vaccination which has reduced the small-pox mortality in England out of all proportion to the protective power of infantile vaccination, and which makes it reasonable to anticipate with confidence that if revaccination in adolescence were added to infantile vaccination, small-pox would disappear, as indeed it has disappeared in Germany.”

**Rapidity of protection gained.**—With regard to the exact period at which immunity to small-pox is produced by vaccination—i.e. on which day after the performance

<sup>1</sup> *St. Bartholomew's Hosp. Journ.*, July, 1903, p. 155.

of the inoculation—it is difficult to be certain. No doubt the immunity is a gradually increasing one, but it is probably slight before the vesicles are well developed, and is mainly brought about from this period to the time when they become purulent. According to Bryce,<sup>1</sup> protection is complete by the fourth day after vaccination, and only a modified small-pox is likely to ensue in cases in which exposure to infection is contemporaneous with vaccination, a fatal issue being improbable in such a case. It will be remembered that the incubation period of small-pox is usually about twelve days, so that vaccinia will have time to develop to its full extent in the interval between infection and the onset of symptoms. According to E. Hart,<sup>2</sup> immunity reaches its maximum about the fourth week after vaccination. There is little doubt that individuals vary as to the rapidity with which protection is gained, as well as with regard to the length of time for which it remains. The “memory” of tissue-cells with regard to the production of immunizing substances is as liable to vary as the mental memory for events.

**Modified small-pox.**—Small-pox occurring in persons who have been vaccinated is generally of the kind known as “modified” small-pox. The eruption is usually scanty and comes out rapidly, becoming vesicular within twelve to twenty-four hours. Some of the papules may never develop into vesicles. The vesicles which do form are often smaller than those seen in the unmodified disease, and many of them dry up without becoming pustular. The crusts fall off more rapidly than in ordinary small-pox, and less pitting is generally left behind. The constitutional symptoms are much less pronounced, and often subside entirely within two or three days, the patient being practically well within a fortnight of the onset. Complications are infrequent and scarcely ever severe.

<sup>1</sup> *Boston Med. and Surg. Journ.*, Feb. 26, 1903.

<sup>2</sup> Allbutt's “*System of Medicine*,” 1897, ii. 578.

## SERUM TREATMENT

**Serum of immune cattle.**—Thomson and Brownlee<sup>1</sup> made experiments with regard to a possible antitoxic influence, upon patients suffering from small-pox, of the serum derived from heifers which were immune to vaccinia. Large quantities of the serum were injected, but the results were apparently quite negative. In certain cases a modified form of the disease occurred; but, as the patients had been vaccinated, it was probably to be attributed to this cause. The serum did not appear to influence the course of vaccination (revaccination) in one case in which this was done.

**Serum of convalescents.**—This has been tried by Tessier and Marie<sup>2</sup> in 13 severe cases of variola, with 8 recoveries. The doses administered were from 25 c.c. to 100 c.c.

**Antistreptococcic serum.**—With a view to diminishing complications, the use of antistreptococcic serum has been suggested (Lindsey). Schoull<sup>3</sup> has made a practice of injecting 60 c.c. of this serum in three doses of 20 c.c., and gives even more than this in severe cases. No pain or reaction is induced by the injection, which is given in the flank, all antiseptic precautions being taken. He claims that rapid improvement results in all the symptoms which are connected with the eruption. The painful condition of the face subsides; photophobia, dysphagia, and hoarseness diminish; pruritus is checked. In some instances a single injection of the antistreptococcic serum produced an immediate fall of temperature. Even hæmorrhagic cases may recover under this treatment. In all Schoull treated 5 hæmorrhagic, 8 confluent, and 9 discrete cases. Out of these, 2 patients died (9 per cent.), whereas the general

<sup>1</sup> *Lancet*, 1903, i. 947.

<sup>2</sup> *Compt. Rend. Acad. Sci.*, 1910, clv. 1536.

<sup>3</sup> *La Semaine Méd.*, March 11, 1903; *Med. News*, April 25, 1903, p. 794,

mortality in cases not so treated was 20·5 per cent. Alfred Smith<sup>1</sup> speaks enthusiastically of this method of treatment, as shortening the duration of the disease and preventing pitting and complications. The serum should be used early, and in sufficient quantities (20 c.c., repeated).

**Salvarsan** has been tried empirically by Sunder<sup>2</sup> in 6 cases of small-pox with apparent benefit.

### CONCLUSIONS

1. Vaccination confers an *active immunity* against small-pox, and protects almost absolutely for a certain period of time. This immunity gradually diminishes, and in many cases disappears after a longer or shorter interval, which varies in different individuals. The immunity may be renewed by revaccination.

2. In a person who has been even once vaccinated small-pox generally occurs, if at all, in a modified form, which is comparatively seldom fatal.

3. If aseptic methods are practised and calf-lymph, duly sterilized, used, the danger of any ill effects resulting is very small indeed. Untoward results are generally due to want of cleanliness and lack of care in the after-treatment of the lesions resulting from the inoculation.

4. Complications in the course of small-pox are generally due to intercurrent infection with various pyogenic organisms, and there is reason to believe that in some instances the use of antistreptococcic serum may prove beneficial in averting or modifying them.

5. Attempts to treat the disease with a serum derived from immune cattle have been unsuccessful. No *antitoxic* serum is known.

<sup>1</sup> *Med. Record*, April 2, 1904, p. 533.

<sup>2</sup> *Arch. f. Schiffs- u. Tropenhyg.*, 1912, xvi. 563.

## CHAPTER X

### ANTHRAX AND GLANDERS

#### ANTHRAX

**Causal agent.**—The *Bacillus anthracis* was the earliest micro-organism to be discovered, on account of its large size, the discovery being made by Davaine in 1850. The organism had probably been seen by Pollender in the previous year.

Of its *toxins* little is known; it does not even seem definitely settled whether it produces a soluble poison or only contains an intracellular toxin.

#### SERUM TREATMENT

**Sclavo's serum.**—After a long course of experiments, Sclavo<sup>1</sup> succeeded in immunizing goats against *B. anthracis*, and found that their serum had a protective and curative effect. The goats were injected first with attenuated cultures of the bacilli, and afterwards with virulent organisms. Rabbits were protected by the serum against lethal doses of anthrax bacilli, and the injection of an adequate dose within twenty-four hours after infection exerted a curative effect. Great difficulty is, however, experienced in preparing a serum sufficiently potent to protect against the more virulent strains of the organism. To test the value of the serum, rabbits are injected intravenously with 5 c.c. of the serum, and then receive, two hours later, one-tenth part of a virulent agar-culture of *B. anthracis*.

More recently Sclavo has made use of asses for the

<sup>1</sup> *Berl. klin. Woch.*, 1901, Nos. 18 and 19, pp. 481, 520.

preparation of his serum. It is found that if 2 c.c. of the serum thus obtained is injected into a rabbit along with 1 c.c. of a fresh, virulent broth-culture of bacilli, the animal is able to survive. For use in man, 30 to 40 c.c. is injected in the flank, the whole amount being divided and injected in three or four different places. In severe cases, intravenous injection may be employed. The administration of the serum may be followed by a rise of temperature, which is of good prognostic import. Sclavo considers that his serum acts by stimulating the leucocytes in their conflict with the germs; in any case it is an antibacterial, not an antitoxic serum.

Very favourable results are recorded in cases of human anthrax in which Sclavo's serum was used. Thus, Cigognani<sup>1</sup> records the successful use of this serum in a series of 14 cases. Large doses were given without any ill effects beyond the appearance of urticaria. The amount used was 40 c.c., which he found it best to give intravenously. Good effects are rapidly produced. The general condition improves in a few hours, and the pustules heal up within two days, while convalescence is much shortened. Cases which appeared alarmingly ill were cured by the use of the serum.

Legge<sup>2</sup> collected 67 cases treated with this serum; in 56 the serum was used alone. He quotes Sclavo's statistics of 143 cases, with a mortality of 6 per cent., as compared with the average mortality of 24 per cent. The serum should be given freely. It may be followed by a sharp rise of temperature (104°–105° F.). Successful cases in this country are recorded by Lockwood and Andrewes,<sup>3</sup> Stretton,<sup>4</sup> and Mitchell.<sup>5</sup> Gutfreund<sup>6</sup> states that the serum

<sup>1</sup> *Gaz. degli Ospedali*, 1902, No. 114.

<sup>2</sup> Milroy Lecture, *Brit. Med. Journ.*, 1905, i. 589.

<sup>3</sup> *Brit. Med. Journ.*, 1905, i. 16.

<sup>4</sup> *Lancet*, 1905, i. 1420.

<sup>5</sup> *Brit. Med. Journ.*, 1905, ii. 118.

<sup>6</sup> *Abstr. in Zeitschr. f. Immunitätsforsch.*, 1910, p. 1087.

is valuable if it be given intravenously, but useless when administered subcutaneously.

**Mendez's serum.**—Mendez<sup>1</sup> has immunized horses by means of injections extending over periods of six to eight months, and finds that he obtains a useful serum. He records that in 25 cases in man the injections were followed by feeling of improvement, with fall of temperature, and subsidence of oedema and glandular swelling. The dose used is 20 c.c., and it is seldom necessary to repeat it. In cattle and sheep the serum has a curative effect, acting efficiently even in doses of 0·5 to 1 c.c.

**Deutsch's serum.**—L. Deutsch<sup>2</sup> has also prepared a serum. It seems to be efficient for treatment of cattle, but has not been used upon man. It agglutinates the bacilli strongly, and contains an immune body or copula capable of destroying the organisms in the presence of a suitable complement.

**Non-specific treatment.**—Bettmann and Laubheimer<sup>3</sup> have used *salvarsan* in cases of anthrax with apparent benefit.

**Vaccines.**—Prophylactic vaccination against anthrax has been carried out in cattle on a large scale with satisfactory results, but as it does not apply to human beings it cannot be dealt with here.

## GLANDERS

**Causal agent.**—The *Bacillus mallei* was discovered by Loeffler and Schutz in 1882. Although primarily a disease of horses, man is highly susceptible—on an average 4 deaths per annum are recorded in England and Wales—and when attacked by the acute form almost invariably succumbs. Eyre<sup>4</sup> states that among 245 collected human cases,

<sup>1</sup> *Centralbl. f. Bakt.*, 1899, xxvi., Nos. 21 and 22.

<sup>2</sup> Deutsch and Feistmantel, "Impfstoffe und Sera." Leipzig, 1903.

<sup>3</sup> *Deut. med. Woch.*, 1912, p. 349.

<sup>4</sup> Art. "Glanders" in "Quain's Dictionary of Medicine," 1902,



including both acute and chronic forms, there was a mortality of 85 per cent. In a series of chronic cases alone Bollinger states that 50 per cent. recovered.

**Toxins.**—By growing the bacilli for a month in flasks of veal-broth at 37° C., then destroying the bacteria by heat and filtering the culture-medium through a porcelain filter, a solution of toxins comparable to old tuberculin is obtained. This is known as malleïn, and is employed in the diagnosis of glanders in animals, but has no place in human medicine.

**Serum.**—No antiserum has been prepared for use in this disease.

**Vaccine treatment.**—Wright<sup>1</sup> has treated one case of chronic glanders successfully with vaccine. We have treated one such case with a vaccine prepared from the patient's own bacilli, with some improvement of the local lesions, but the malleïn reaction at the site of injection of the vaccine and the rise of temperature were so marked that the patient soon refused to continue the treatment and discharged himself from the hospital.

**Agglutination reaction.**—As *B. mallei* is agglutinated by the serum of infected animals and man, this reaction is valuable as a test for the presence of the disease. The microscopical method of observing agglutination, which is so reliable in the case of enteric fever, is, however, valueless here, as the reaction is tardy and the results are inconsistent; but with the macroscopical reaction the results are quite trustworthy. For this purpose various dilutions of the suspected serum are made with normal saline solution by means of a graduated pipette; a homogeneous emulsion in sterile water, or preferably, on account of the virulence of the organism, in 1 per cent. formalin solution, of an agar-culture of *B. mallei*, twenty-four hours old, is also prepared, and equal quantities of the emulsion and of each of the dilutions are mixed either in small test-tubes or taken up in capillary pipettes and sealed. Control preparations

<sup>1</sup> "Studies on Immunization," 1909, p. 406.

with 1 : 5 and 1 : 10 solution of normal serum are likewise put up for comparison. The preparations are allowed to stand at room-temperature for twelve to eighteen hours ; at the end of the time they are examined, and those dilutions in which complete sedimentation of the bacilli has taken place are noted. Normal serum, even in 1 : 5 dilution, fails to produce sedimentation of the bacillus, whilst the serum of an individual infected with glanders gives a good reaction in 1 : 50 dilution, in many cases in 1 : 100, and sometimes in 1 : 200 and even higher dilutions.

## CHAPTER XI

### PLAGUE

**Causal organism.**—The *Bacillus pestis* was discovered by Yersin in 1894. The bodies of the bacteria themselves are highly toxic, but the organism does not appear to form virulent poisons in culture-media. Klein's experiments, however, seem to show that some toxic material is contained in the fluid of broth-cultures of the bacilli (*see* p. 209); and Dean,<sup>1</sup> who also obtained evidence of the existence of free toxin in old cultures, showed that it could be separated from the bacilli by filtration. By its action as a parasite, the organism produces a "hæmorrhagic septicæmia," that is to say, a general infection (the organisms multiplying in the blood-stream), with interstitial hæmorrhages in the various organs. Curative serums have been prepared for the treatment of the disease, and protective vaccination has been carried out.

### DIAGNOSIS

**Agglutination.**—Plague bacilli, like those of enteric fever and many others, are agglutinated by the serum of patients who have just suffered from the disease, or of animals which have been inoculated with the bacilli or their products. There is some difficulty in performing the test owing to the normal occurrence of the bacilli on nutrient media in closely adherent masses; if, however, the emulsion is made in sterile water and well shaken with glass beads,

<sup>1</sup> "Studies in Pathology, in celebration of the Quatercentenary of the University of Aberdeen," 1906.

the bacilli become thoroughly separated. Klein<sup>1</sup> advises that they should be grown on gelatin, on which a drier and less sticky culture is formed, and that they should then be suspended in salt-solution. If it be found that the bacilli are still present in clumps and not distributed singly, it is better to make a thick emulsion of them, and to filter it through a double thickness of filter-paper. The microscopic method of examination must be applied, as the naked-eye or "sedimentation" test is unreliable. Emulsions of the bacilli in broth are also to be avoided for this test, as they tend to spontaneous agglutination without the aid of immune serum. Klein recommends a dilution of 1 : 20 for use, and a time-limit of half an hour. He finds that the blood of immunized animals, though strongly agglutinative, is not bactericidal. The growth of *B. pestis* is said to be retarded by addition of serum from a plague convalescent ; this appears to contain a bacteriolytic copula.<sup>2</sup>

In human patients the agglutinative power of the blood does not develop until late in the disease, often not till convalescence is established. The test is therefore useless clinically. It may, however, be valuable as a proof that a specimen of bacillus under examination is *B. pestis*.

#### HAFFKINE'S PROPHYLACTIC

**Preparation of vaccine.**—Haffkine prepares his vaccine by growing *B. pestis* in flasks of broth to which a few drops of fat or oil have been added. Each drop, as it floats on the surface of the liquid, acts as a focus for the development of the organisms, which form colonies hanging down into the broth in the shape of stalactites. The vessels are shaken from time to time, by which means the hanging colonies are thrown down into the fluid, and others form in their places. When growth has gone on for a month or six weeks, the bacilli are killed by heating to 70° C. for one to

<sup>1</sup> *Thirty-first Annual Report of L.G.B.*, 1901-2; Supplement containing Report of Medical Officer, Appendix B, No. 1, p. 361.

<sup>2</sup> Low, *Trans. Grant Coll. Med. Soc.*, Bombay, 1903-4, p. 3.

three hours; the fluid is tested by culture to make certain that it is sterile, after which it is ready for use as vaccine. The usual dose for an adult man is 3 c.c., for a woman rather less (2 to  $2\frac{1}{2}$  c.c.); children receive still smaller amounts. The vaccine is given by subcutaneous injection, and its administration is followed by redness and swelling at the seat of inoculation, with constitutional symptoms in the form of rise of temperature and feeling of illness. The latter pass off in about twenty-four hours, but the patient should spend the first day after the treatment at rest, not resuming his ordinary avocations till the second day.

**Results of inoculation.**—Haffkine considers that protection against plague is produced rapidly—at the end of twenty-four hours. In view of the facts ascertained by Wright as to antityphoid inoculation, it seems likely that there may be at first a period of increased susceptibility to infection, and this has been asserted by Calmette. Bannermann, however, denies that this is the case, and considers that the injection does not aggravate an attack, if made during the incubation period. Of the figures given by Haffkine as to the results obtained with his inoculations, we may quote those relating to the village of Undhera.<sup>1</sup> Among 64 uninoculated persons there were 27 cases of plague, and 26 of these proved fatal; while among 71 inoculated persons—members of the same families as the former and living under exactly the same conditions—there were 8 cases, 3 of which were fatal. The deaths among the uninoculated thus exceeded those among the inoculated by 89·65 per cent.

Leuman<sup>2</sup> records that of 1,173 mill-hands, 1,040 were inoculated twice: among these there were 22 deaths (2·11 per cent.); of 58 inoculated once, 8 died (13·79 per cent.); of 75 not inoculated, 20 died (26·6 per cent.). Bannermann states that in a total of 6,000 cases the mortality among the

<sup>1</sup> *Lancet*, 1899, i. 1697.

<sup>2</sup> Quoted by Miss Slaughter, *Johns Hopkins Hosp. Bull.*, Nov., 1903, p. 307.

inoculated was 43·5 per cent., while among the uninoculated it was 73·7 per cent.

Among 7,182 inoculated municipal labourers,<sup>1</sup> 14 cases of plague occurred (0·19 per cent.) with 13 deaths (0·18 per cent.); whereas among 418 uninoculated there were 28 cases (6·7 per cent.) and 26 deaths (6·2 per cent.).

Haffkine<sup>2</sup> sums up the results so far obtained in the following figures:—*Inoculated*, 186,797 : 3,399, or 1·8 per cent., attacked with plague ; 814, or 0·4 per cent., died. *Uninoculated*, 639,630 : attacks, 49,433, or 7·7 per cent. ; deaths, 29,733, or 4·7 per cent

The Indian Plague Commission reported as follows with regard to this method of prophylaxis:—

(1) Inoculation sensibly diminishes the incidence of attacks of plague. It is, however, not an absolute protection against the disease.

(2) The death-rate is markedly diminished by its means, not only the incidence of the disease but also the fatality (case-mortality) being reduced.

(3) The protection is not conferred, on those inoculated, for the first few days after the injection.

(4) The duration of the immunity is uncertain, but it seems to last for a number of weeks, if not for months.

The mode of action of Haffkine's prophylactic is presumably the same as that of other vaccines, viz. it depends for its efficacy on the presence of the actual bacteria contained in it. It has, therefore, generally been supposed that the precipitate that forms in tubes of the vaccine which are allowed to stand, consisting of the bodies of the dead bacteria, is the effective part of the preparation. Klein<sup>3</sup> has thrown some doubt on the inert nature of the supernatant fluid. Probably it contains some dissolved bacterial proteins. He finds that it has a certain, though

<sup>1</sup> *Ann. Rept. Sanitary Commissioner*, 1905.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1906, iv. 825.

<sup>3</sup> *Thirty-first Ann. Rept. of the Local Govt. Board*, 1901-2 ; Supplement containing the Report of the Medical Officer, 1903, pp. 357-94.

small, protective influence on rats. Further, he finds that the blood of immunized animals is agglutinative towards the *B. pestis*, but not bactericidal.

The following is the judgment set forth in the *Annual Report of the Sanitary Commissioner with the Government of India* for 1904 : "That its value is great is certain ; not only does it largely diminish the danger of plague being contracted, but, if it fails to prevent the attack, the probability of a fatal event is reduced by one-half" (p. 107). In the Report for the following year (1905) it is stated that the use of the prophylactic has no ill effect on health, and that a marked protective influence lasts for six or even twelve months (p. 122).

Wurtz and Bourges,<sup>1</sup> from experiments on white mice, find that the protective power of the prophylactic is considerable, and lasts for a moderate period of time (two or three months). Haffkine considers that the protection afforded by his prophylactic lasts as long as six months. The general opinion in India is that it is "absolutely safe for three months." Leuman found that the protection gained by those twice inoculated was 10 per cent. greater than that of the once-inoculated.

Pfeiffer<sup>2</sup> considers that the bacilli lose some of their virulence by being cultivated in broth, and that their efficacy as a protective is thus diminished. He has accordingly prepared a vaccine from fresh cultures of *B. pestis* on agar. These are emulsified in broth or salt-solution, and sterilized at 65° C. The reaction produced by injection of Pfeiffer's preparations is more intense than that seen after Haffkine's prophylactic. No statistics are available for forming a judgment as to the value of this vaccine as compared with Haffkine's.

Kolle and Strong<sup>3</sup> suggest vaccination with living

<sup>1</sup> *Arch. de Méd. Expérimentale*, etc., 1902, p. 145.

<sup>2</sup> *Deut. med. Woch.*, March 15, 1906, p. 413.

<sup>3</sup> Quoted by Marx, "Diagnostik, Serumtherapie und Prophylaxe," p. 81.



attenuated bacilli, and Strong has actually carried out this method at Manila without any untoward occurrences.

#### TERNI AND BANDI'S VACCINE

Terni and Bandi<sup>1</sup> prepare a special material, for use as a vaccine against plague, by injecting guineapigs intraperitoneally with plague bacilli and collecting the inflammatory fluid which is secreted into the peritoneal cavities of the animals. This fluid is sterilized by heating for a short period of time, on each of several consecutive days, to 50° C., and is preserved by the addition of a small proportion of carbolic acid. The inventors claim that by means of this vaccine immunity may be produced in eight to ten hours, and that the blood of persons so treated possesses bactericidal powers.

Havelburg<sup>2</sup> records that this vaccine was used with good effects in Brazil. Pinto<sup>3</sup> also records good results with anti-plague vaccinations (with this remedy?): out of 1,803 persons vaccinated only 2 contracted plague, and one of these cases occurred immediately after the vaccination. He considers the results of the treatment to be brilliant, but it must be remembered that the plague in Brazil was apparently of a mild type. Kolle and Otto<sup>4</sup> regard Terni and Bandi's vaccine as quite inert.

#### LUSTIG AND GALEOTTI'S VACCINE

The material used for the preparation of Lustig's serum (p. 216) may be employed for the purpose of vaccinating against the disease. It is prepared by growing the bacilli in broth and then on agar. The bacteria are washed off

<sup>1</sup> *Deut. med. Woch.*, 1901.

<sup>2</sup> *Berlin. klin. Woch.*, 1901.

<sup>3</sup> Abst. in *Journ. of the American Med. Assoc.*, 1902, i. 681. The nature of the vaccine used is not stated in the abstract. We have been unable to obtain the original article (*Tidsskrift f. d. Norske Lægeforen.*, Feb. 1, 1902).

<sup>4</sup> *Deut. med. Woch.*, July 9, 1903.

and dissolved in a 1-per-cent. solution of caustic potash, and the fluid is neutralized with 1-per-cent. acetic acid. A precipitate is thus formed, which is highly toxic, containing as it does the intracellular poisons of the bacilli. It is dried *in vacuo*, and can be readily preserved in this form. For use as a vaccine, it is dissolved in a weak solution (1 or 2 per cent.) of sodium carbonate. The dose for an adult is 0.0133 grm. of solid substance. Two grm. of the solid dissolved in 1 litre of solution will afford material for 143 vaccinations.<sup>1</sup> Statistics as to the use of this vaccine are not available.

Tavel, Krumbein and Glucksmann<sup>2</sup> grew broth-cultures by Haffkine's method, and precipitated them after a month's growth by ammonium sulphate. The precipitate was extracted with 1-per-cent. caustic soda, and the nucleo-protein thrown down with acetic acid. The precipitate is dried *in vacuo* and stored as a powder. When required for use the requisite dose is dissolved in 1-per-cent. sodium carbonate solution.

#### OTHER VACCINES

Klein<sup>3</sup> has produced immunity in animals by injection of an emulsion of the dried organs of an animal dead of plague, an average guineapig yielding 5-7 grm. of dried powder, equivalent to 400-600 doses for an adult rat. A very similar procedure has been devised by Wallannah.<sup>4</sup> Besredka<sup>5</sup> advises the use of bacilli agglutinated (sensitized) with antiplague serum, then killed by heat, and finally suspended in salt-solution. In a later communication<sup>6</sup> this observer described the use of normal horse-serum as sensitizer instead of immune serum.

<sup>1</sup> Deutsch and Feistmantel, "Impstoffe und Sera." Leipzig, 1903.

<sup>2</sup> *Zeitschr. f. Hyg.*, 1902, xl. 239.

<sup>3</sup> See *Brit. Med. Journ.*, 1906, i. 155.

<sup>4</sup> *Ann. de l'Inst. Pasteur*, 1905, xix. 589; also *Lancet*, 1907, i. 222.

<sup>5</sup> *Ibid.*, 1902, xvi. 918.

<sup>6</sup> *Ibid.*, 1905, xix. 479.

## YERSIN'S SERUM

**Preparation of the serum.**—The original method of Yersin, Calmette and Borel<sup>1</sup> for the preparation of anti-plague serum was by inoculation of horses with fresh agar-cultures of the bacilli. It was subsequently found by Roux and Wladimiroff that as effective a serum could be obtained by injection of cultures killed by heating to 50° C. for one hour, by which proceeding the danger attending the use of living organisms could be avoided. Kolle<sup>2</sup> used suspensions heated to 65° C. for several hours. The serum is difficult to prepare of adequate strength, and attempts at its manufacture are at times unsuccessful. Krumbein, Tavel and Glucksmann<sup>3</sup> took a year and a half in attaining a sufficiently active serum. Six months is the time usually found necessary for the preparation of the serum at the Paris Institute. Before the serum is finally drawn off for use, the blood of the horse is tested on mice to ascertain that no living bacilli are contained in it. One-tenth of a cubic centimetre of serum should protect a mouse from a dose of living bacilli which kills a control mouse in two or three days.

**Value of Yersin's serum.**—Yersin gives the following account of his experiences in Amoy.<sup>4</sup> Twenty-three cases were treated in all. Of these—

Six cases treated on the first day ; all recovered within 24 hours. Dose, 20–30 c.c. No suppuration occurred.

Six cases treated on the second day ; all recovered within 3–4 days. Dose, 30–50 c.c. No suppuration.

Four cases treated on the third day ; all recovered within 4–5 days. Dose, 40–60 c.c. Two suppurated.

Three cases treated on the fourth day ; all recovered within 5–6 days. Dose, 20–50 c.c. One suppurated.

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1895, ix. 590.

<sup>2</sup> *Deut. med. Woch.*, 1897.

<sup>3</sup> *Centralbl. f. Bakt.*, 1901, xxx. 742.

<sup>4</sup> *Ann. de l'Inst. Pasteur*, 1897, xi. 81.

Four cases treated on the fifth day, two died. Dose, 60-90 c.c.

In Nhatrang (Annam),<sup>1</sup> out of 33 cases treated with the serum, 19 recovered and 14 died (mortality, 42 per cent.); of 39 cases not treated, all died (100 per cent.).

Calmette and Salimbeni<sup>2</sup> used the serum in Oporto. They report that, of 142 cases injected with the serum, 24 died, a mortality of 14.78 per cent.; among 72 patients not so treated, 46 died, a death-rate of 63.72 per cent. They find that the serum reduces the pain in the bubo and limits the inflammation; suppuration is often aborted by its early use.

Cairns,<sup>3</sup> as the result of experience of the remedy in cases at Glasgow, concludes that—

(1) Yersin's serum is a remedy of the greatest value.

(2) Its action is bactericidal—as shown by the degeneration induced in the bacilli—as well as antitoxic.

(3) Good results are best secured by the early administration of large doses, subcutaneously, into the area from which lymph drains towards the bubo, and also intravenously.

(4) In mild cases the subcutaneous method alone is sufficient, but in severe attacks combined subcutaneous and intravenous administration is advisable. The total combined dose in the latter condition should be 150 to 300 c.c., the proportion given intravenously varying with the severity of the attack.

Valassopoulo<sup>4</sup> used the serum supplied by the Pasteur Institute (Paris) in 100 cases; among 64 treated during the first three days of the disease the mortality was 10 per cent.; among 36 treated on the fourth day and later it was 47 per cent. The mortality among 42 cases not treated with serum was 35 per cent., but in this class were included many very mild attacks. Valassopoulo agrees with Roux

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1899, xiii. 251.

<sup>2</sup> *Ibid.*, 1899, xiii. 865.

<sup>3</sup> *Lancet*, 1903, i. 1287.

<sup>4</sup> *Bull. Soc. Méd. des Hôp.*, 1908, p. 541.

that the serum is antitoxic, not bactericidal. Finally, the Indian Plague Commission<sup>1</sup> concluded that neither Yersin's nor Rowland's serum was effectual in septicæmic cases, and that in bubonic cases their value was doubtful.

**Dose and administration of the serum.**—From what has just been said it may be seen that large doses of the serum are to be employed, if the amount is available. Yersin appears to give doses of 20 to 90 c.c. according to the date at which the case comes under treatment. He administers the remedy subcutaneously. Cairns used still larger amounts (150 to 300 c.c.), and gave the serum both subcutaneously and intravenously; and the advantages of employing large doses are also enforced by Duprat.<sup>2</sup> Brownlee<sup>3</sup> insists on the intravenous use of the serum, and advises doses of 60 c.c.; Lignières<sup>4</sup> gives the same advice. There is no reason to fear the use of the larger amounts. The only ill effects recorded have been pains in the joints and erythema, noted by Calmette and Salimbeni, analogous to those associated with diphtherial and other antitoxins.

Choksy<sup>5</sup> advises an initial injection of 100 c.c., followed by two others of the same amount at intervals of six to eight hours. These may be supplemented by subsequent injections of smaller quantities.

Denys and Tartakovsky<sup>6</sup> insist on the importance of local injections of the serum into the neighbourhood of the buboes. Thus, in cases of inguinal buboes, the remedy should be injected into the leg. They found that if guinea-pigs were inoculated intraperitoneally with plague bacilli, 0·1 c.c. of serum injected into the peritoneal cavity would act as a protective dose; whereas 10 c.c. administered

<sup>1</sup> *Journ. of Hygiene*, 1912, xii. Suppl. II., 326.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1903, xvii. 599.

<sup>3</sup> *Lancet*, 1901, ii. 435.

<sup>4</sup> *Ann. de l'Inst. Pasteur*, 1901, xv. 808.

<sup>5</sup> *Rept. on Treatment of Plague*, Bombay, 1906.

<sup>6</sup> *Semaine Méd.*, 1900, p. 40.

subcutaneously was of no avail in saving the lives of the animals.

**Prophylactic use of the serum.**—Yersin records that in Nhatrang no cases of plague occurred among those who had received prophylactic injections of the serum. Calmette and Salimbeni also used the serum as a protective, giving doses of 5 c.c. injected under the skin of the abdomen. According to these observers the protection only lasts fifteen days, so that it is advisable to repeat the injections at the end of this time. Calmette recommends the injection of some of the serum along with the use of Haffkine's prophylactic, in order to counteract the first depressing effects of the latter. This suggestion seems worthy of serious consideration when the prophylactic is used in the actual presence of an epidemic. Serum-rashes, joint-pains, and hyperidrosis may follow the use of this serum.

#### LUSTIG'S SERUM

**Antitoxic serum.**—Yersin's serum appears to be bactericidal in nature, though it may possess some antitoxic power. Lustig considers that the curative serum for plague should be mainly antitoxic, and he therefore proceeds to obtain such a preparation by immunizing horses with the vaccine material already described (p. 211), which consists of a poisonous bacterio-protein. The immunizing process lasts two or three weeks.

Lustig and Galeotti<sup>1</sup> record that among 475 cases of plague treated with the serum the recovery-rate was 39·36 per cent., whereas among 5,952 patients not so treated the recoveries were only 20·6 per cent. Choksy<sup>2</sup> puts the rate of recovery after use of the serum at 38·2 per cent., while in other patients not so treated it was only 19·5 per cent. In another series of cases, 480 patients were treated with the serum, and the same number without it. Eliminating various sources of error, he found that the recoveries among

<sup>1</sup> *Brit. Med. Journ.*, 1901, i. 206.

*Lancet*, 1900, ii. 291.

the serum cases amounted to 39·62 per cent., whereas among the non-injected cases they were only 20·21 per cent. The following table shows the results obtained in India with this remedy (Choksy)<sup>1</sup> :—

TABLE SHOWING RESULTS OF TREATMENT OF PLAGUE WITH LUSTIG'S SERUM. BOMBAY, 1898-1901

Period	Serum-treated patients			Patients under ordinary treatment			Difference in favour of the serum-patients percent.
	No.	Deaths	Case-mortality, per cent.	No.	Deaths	Case-mortality, per cent.	
May to Oct., 1898...	257	145	56·4	752	595	79·1	22·7
Jan. to April and June, 1899... ..	189	124	65·60	884	734	83·03	17·4
May, 1899, and July, 1899, to Aug., 1900	484	329	68·00	484	385	79·5	11·5
Aug., 1900, to Feb., 1901 (3 extra cases) ... ..	55	36	65·45	184	144	78·26	12·81
March, April, and May, 1901 ... ..	104	81	77·82	102	81	79·42	1·53

Mayr<sup>2</sup> gives an account of 361 cases treated with the serum, the recovery-rate being 33·8 per cent.; while among patients treated by other methods only 21·3 per cent. survived. He says that the general recovery-rate in hospitals where the serum was used was 4·5 per cent. higher than in those where it was not employed. He considers that the curative properties of the serum are definitely established.

<sup>1</sup> "The Treatment of Plague with Professor Lustig's Serum," p. 110. Bombay, 1903.

<sup>2</sup> *Lancet*, 1900, ii. 461.



A mere study of the above records does not produce a very favourable impression of the value of the remedy. The results obtained do not seem so striking as those seen with Yersin's preparation. More experience is, however, needed to enable us to form a judgment. The opinions of those who have used Lustig's serum, as quoted above appear to be favourable, and Choksy,<sup>1</sup> in his most recent statistics, states that the use of Lustig's serum reduces the mortality from 74·5 per cent. to 10·5 per cent. He gives an initial dose of 100 c.c. followed by similar amounts at twelve and twenty-four hours.

**Rowlands and MacConkey's serum.**—Rowlands<sup>2</sup> prepared a vaccine by mixing the growth from the surface of agar with a suitable quantity of anhydrous sodium sulphate. This was used by MacConkey<sup>3</sup> in immunizing horses, but the serum of these animals did not reach a very high antitoxic value (*see* p. 215).

## CONCLUSIONS

1. *Haffkine's prophylactic* is a valuable means of protection against plague. There is some doubt as to whether its use in the presence of an epidemic is advisable, owing to the possibility of an increased susceptibility being at first produced. The employment of some of Yersin's serum along with the vaccine seems to offer a means of counteracting this depressing effect, if it really exist.

2. Sufficient evidence is not yet available to enable us to decide as to the efficacy of *Lustig's* or of *Terni and Bandi's vaccine*.

3. *Yersin's serum* is of value as a remedy for the disease. It should be given early in the case and in large quantities. Some of the serum should be injected intravenously, the rest subcutaneously into the area of skin which is drained

<sup>1</sup> *Zeitschr. f. Immunitätsforsch.*, 1912, p. 1074.

<sup>2</sup> *Journ. of Hygiene*, 1910, x. 536.

<sup>3</sup> *Ibid.*, Plague Suppl. II. 1912, p. 387.

by the lymphatics leading to the bubo. The dose may be from 60 to 150 or even 300 c.c.

4. Yersin's serum may also be used *prophylactically* (dose 5 to 10 c.c.), but the protection gained is transitory, so that repeated injections are necessary in presence of an epidemic of plague.

5. The claims of *Lustig's serum* as a remedy are less well established than those of Yersin's serum, but some evidence has been adduced in its favour.

## CHAPTER XII

### CHOLERA

**Causal organism.**—The organism which is responsible for the production of cholera is a curved, rod-shaped bacterium, which was discovered by Koch, and is called the *Vibrio cholerae*, or *Spirillum cholerae*.

**Toxins.**—It has not been possible to prepare any potent solutions of the toxins of the organisms in artificial media: the toxins are therefore classed as “intracellular.” Ransom,<sup>1</sup> however, obtained from cultures a solid substance which induced the formation of an antitoxin when injected into goats. Metchnikoff and his assistants also succeeded in obtaining a feebly toxic fluid by growing the vibrios in peptone-water. These preparations can, however, only faintly reproduce the toxins which are manufactured in some forms of the disease, of which the virulence is such that, in acute cases, death may occur within a few hours. The bodies of the vibrios contain within them a substance which produces necrosis of tissue when they are injected subcutaneously.

Macfadyen<sup>2</sup> obtained an antitoxic serum by injecting rabbits and goats with ground-up cultures of the vibrios; it was also agglutinative and bacteriolytic. The endotoxin of the bacteria is thermolabile, being destroyed at a temperature of 55° to 60° C.

Allusion has already been made to Pfeiffer's experiments (p. 9) on the destruction of cholera vibrios in the

<sup>1</sup> *Deut. med. Woch.*, 1895.

<sup>2</sup> *Lancet*, 1906, ii. 494. Cf. Brau and Denier, *Ann. de l'Inst. Pasteur*, 1906, xx. 578; Hahn, *Münch. med. Woch.*, 1906, No. 23.

peritoneal cavity of immune animals. It is found, however, that if a large dose of the bacteria is injected into an "immune" guineapig, the animal dies in spite of the destruction of the organisms; in other words, although the bacteria are broken up and killed by the serum, this has no power of neutralizing their intracellular toxins, which are set free and kill the guineapig.

These experiments on animals are of considerable importance from the point of view of human therapeutics. They show that there can be little hope of treating the disease, when already developed, by means of a bactericidal serum; for if the bodies of the bacteria are so toxic in themselves, such a serum, by leading to rapid breaking-up of those vibrios which were already present, could but produce speedier intoxication. The hope of preparing an antitoxic serum is at present slight, as attempts to produce a potent toxin in artificial media have failed, and it is only by means of a toxin of high potency that an effective antitoxic serum can be obtained. Hence it is in the direction of prophylaxis—of inducing a condition of active immunity which shall destroy the bacteria that first gain access to the body, before they have increased in numbers up to a dangerous degree—that the best hope of combating the disease seems to lie.

### DIAGNOSIS

**Agglutination.**—The cholera vibrios are agglutinated by means of the serum of convalescents from the disease, and by that of animals artificially immunized. Karwacki<sup>1</sup> states that agglutination of the vibrios with the serum of a suspected case in a dilution of 1 : 30 is a satisfactory proof of cholera. The reaction is of no use for the diagnosis of cholera, as it does not occur till late in the disease; it may, however, be employed for the identification of a particular vibrio as that of cholera Asiatica, since there are several organisms which belong to the same group and closely

<sup>1</sup> *Zeitschr. f. Hyg.*, 1906, p. 39.

resemble one another. A more certain test is afforded by Pfeiffer's experiment: the organisms in question are injected into the peritoneal cavity of a guineapig along with a sufficient quantity of a serum known to be bactericidal towards the *V. cholerae*, and their fate is investigated. A serum of the above nature may be readily produced by injecting a rabbit subcutaneously with laboratory cultures of the cholera organisms, and may be preserved by means of 0.5 per cent. carbolic-acid solution.

"Immune" serum is capable of dissolving *V. cholerae* in a test-tube, when it is fresh, but it rapidly loses the power; this, however, may be regained by the addition of a little fresh normal serum of the same species of animal. In other words, the complement or alexine tends to disappear in course of time, whereas the copula or immune body remains stable.

**Complement-fixation.**—Arnako and Kozima<sup>1</sup> have found a positive complement-fixation reaction, using as antigen either the fluid of typical rice-water stools or a culture of the vibrios in peptone-water.

#### VACCINATION AGAINST CHOLERA

Experimental vaccination against cholera was first carried out by Ferran, who employed broth-cultures of the *Vibrio cholerae* derived from the stools of patients. No definite statistics are available as to the amount of success which Ferran obtained by his inoculations. The methods in use at the present time for anticholera vaccination are that of Haffkine and that of Kolle.

**Haffkine's cholera vaccine.**—This method involves the use of two vaccines, a weaker and a stronger, designated respectively I. and II., the former being administered first in order to avoid the destructive effect of the virulent organisms on the tissues at the point of injection. Neither of these vaccines is artificially devitalized. The weaker vaccine is obtained by growing the bacteria on agar at a

<sup>1</sup> *Zeitschr. f. Chemotherap.*, 1912, Orig., Bd. i., Heft 1.

temperature of 39° C. in a current of air. The stronger vaccine is prepared by passing the vibrios through a series of guineapigs till a virus is obtained which is invariably fatal to these animals within eight hours. The method adopted is as follows: A guineapig is inoculated intraperitoneally with a laboratory culture of the organisms, which usually causes death within twenty-four hours. The peritoneal exudate is collected from the dead guineapig, and is incubated for ten hours at 35° C., the optimum temperature for the organisms. (This is done in order to give the bacteria time to multiply, as they are only found in comparatively small numbers in the first peritoneal fluid.) After incubation the fluid from the first guineapig is injected into the peritoneum of a second, and so on through a series of animals, till the "*virus fixe*" is obtained. This is cultivated for twenty-four hours on agar-tubes, the whole surface of a sloped agar-tube being inoculated. When growth has occurred, the whole culture is washed off with sterile broth, and the quantity made up to 8 c.c. One cubic centimetre (one-eighth part) of this constitutes a dose of the vaccine. The virulent cultures soon become attenuated by growth on laboratory media, and must be again raised in virulence by passage through animals.

The injection is given hypodermically in the flank, and an interval of five days should separate the two vaccinations. The procedure is followed by redness, swelling, and pain in the side, and by a febrile reaction. The degree of protection afforded is said to be proportional to the severity of the symptoms. The immunity conferred by each injection is attained in five days; hence the selection of this interval of time between the injections. Before employing the vaccines generally, Haffkine made trial of them on himself and others: no ill effects were produced. He has now given 70,000 injections in 42,179 individuals without accident.

**Kolle's vaccine.**—This is an emulsion of the killed bacteria, and is derived from agar-cultures of a virulent

strain of the cholera virus sterilized at a temperature of 58° C., standardized to contain 4 mg. of the culture in 1 c.c., and preserved with 0·5 per cent. phenol. A first injection of 0·5 c.c. of the vaccine is followed eight days later by a second of 1 c.c.

The injection is followed by local redness and swelling, and a rise of temperature often occurs, which, however, passes off within forty-eight hours.

**Results of vaccination.**—Haffkine<sup>1</sup> gives the following figures relating to his experiences at Calcutta and Lucknow :—

POPULATION				CASES		DEATHS	
				Total	Percentage	Total	Percentage
Non-inoculated	1735	...		174	10·63	113	6·51
Inoculated	...	500	...	21	4·20	19	3·80

Powell<sup>2</sup> also reports favourably on the results obtained with this prophylactic, and gives the following statistics :—

POPULATION			CASES		DEATHS		
			Total	Percentage	Total	Percentage	Fatality
Non-inoculated	6,549		198	3·02	124	1·89	63 %
Inoculated	.. 5,778		27	0·48	14	0·24	50 %

Out of 275 uninoculated coolies on steamers plying between Goalundo and Dilrugarh, 8·36 per cent. contracted cholera, and 10 died; while of 414 who had been inoculated, only 1·2 per cent. contracted the disease, and none died of it.<sup>3</sup>

In 1903 the figures were as follows :—2,633 non-inocu-

<sup>1</sup> *Brit. Med. Journ.*, 1895, i. 219.

<sup>2</sup> *Journ. of Trop. Med.*, 1899, No. 2.

<sup>3</sup> *Ann. Rept. of the Sanit. Commissioner with the Govt. of India*, 1901, p. 88.



lated, 68 cases, 16 deaths; 199 inoculated, 4 cases, 1 death. In 1904, 614 non-inoculated, 6 cases, 3 deaths; 75 inoculated, no case. The inoculations have, nevertheless, been discontinued by the wisdom of the Government.<sup>1</sup>

Savas<sup>2</sup> reports on the excellent results obtained in a large body of troops (114,803), and his figures may be summarized thus:—

POPULATION	CASES		DEATHS	
	Total	Percentage	Total	Percentage
Non-inoculated ... 8,968 ...	834	9·0	166	27
Inoculated once ... 14,613 ...	618	4·0	74	12
Inoculated twice... 91,224 ...	644	0·7	65	10

Cardamatis<sup>3</sup> gives the following figures with regard to an epidemic of cholera in the island of Chalcidice:—

POPULATION	CASES		DEATHS	
	Total	Percentage	Total	Percentage
Before inoculation was introduced... ... 14,256	300	2·10	170	56·66
After one inoculation 14,086	38	0·26	15	39·47
After two inoculations 14,071	2	0·01	0	0

Part of the above reductions in incidence and mortality may have been due to measures of isolation and disinfection, and possibly part to diminution in the virulence of the epidemic, but the figures are striking. Cardamatis emphasizes the fact that inoculation, even with two doses of vaccine, is not an absolute preventive of the disease. He also records as a by-effect of the procedure menstrual disturbances in women—either retardation of the onset of the period or cessation of the flow—if vaccination was

<sup>1</sup> *Ann. Rept. of the Sanit. Commissioner with the Govt. of India*, 1903 and 1904.

<sup>2</sup> *Wien. klin. Woch.*, 1914, p. 1094.

<sup>3</sup> *Bull. Soc. Path. Exot.*, 1914, vii, 447.

performed during its activity; or the incidence of uterine pain. Menorrhagia was seldom produced.

It appears from these figures that the use of Haffkine's prophylactic inoculations confers a certain measure of immunity to cholera. Larger statistical material is necessary to enable us to gauge with accuracy the exact amount of protection which it affords.

There is reason to believe that this, like other methods of vaccination with pathogenic bacteria, may produce an initial fall in the resistance of the individual to the disease; and therefore it may be questioned whether it would be wise to undergo inoculation in the presence of an epidemic. Complete immunity—so far as it is ever complete—is gained at the end of ten days, in which time two vaccinations have been carried out. Those who are about to visit an infected area, but who can allow this period of time to elapse before they are actually brought face to face with the epidemic, would be wise to undergo vaccination. It is possible that a certain measure of protection may be gained by the end of the fifth day, as a result of the first inoculation; but this is probably slight. It is noteworthy that the prophylactic diminishes the liability of the inoculated person to contract cholera, rather than the fatality of the disease when it occurs in those who have been vaccinated.

Marx points out that an element of doubt exists in the statistics of cholera vaccination, in that it is the better-informed upper classes who submit to inoculation, while the poorer portion of the population, owing to ignorance and superstition, refuse to avail themselves of the protection offered. It is among the latter that the incidence of the disease may be expected to be greatest and the mortality highest, so that the apparent protection afforded to the vaccinated may really be due to their better circumstances in other respects.

Fear has been expressed that the necrosing action of the bacilli may act injuriously when they are used as a

vaccine. Kolle,<sup>1</sup> however, believes that this danger may be neglected in practice, and that no ill effects may be expected from an infection of virulent organisms without preparatory inoculation with attenuated cultures. He therefore at first advised only one vaccination, with the virulent organisms, thus producing more rapid immunization. Powell records that Haffkine now uses more virulent cultures for his first vaccination, and that no suppuration or other accident has been noted as a result of this procedure.

Strong<sup>2</sup> devised a mode of vaccination against cholera by means of the body-substance of the vibrios, which he calls "cholera receptors." The vaccine is obtained by "autolytic digestion" of the organisms, i.e. by incubating an emulsion of them in sterile water, in which they break up spontaneously. The following are the directions given by the writer :—

"The surface of flat-sided flasks filled with cholera agar (*sic*) are sprayed with 20-hour bouillon cultures, and the flasks then put aside in the incubator at 37° C. for 20 hours; the growth is then emulsified with sterile water, removed from the surface of the agar, and the emulsion placed in a sterile flask and kept at a temperature of 60° C. for 24 hours. The mixture is then put aside in the incubator for from 2 to 5 days. The best results were obtained apparently after 5 days' autolytic digestion. After such digestion the emulsion is filtered through a Reichel filter. The fluid thus obtained must, of course, be examined for sterility and carefully standardized before being used as a human vaccine."

No trial of the method on man has apparently been made. Strong considers that the disagreeable effects inseparable from the use of Haffkine's vaccine render it unsuitable for general use.

Murata,<sup>3</sup> who inoculated with dead organisms, gives the following figures :—

Inoculated, 10,000, with 6 attacks and 42 per cent.

<sup>1</sup> *Zeitschr. f. Hygiene*, 1894, Bd. xvi. and xviii.; and *Centralbl. f. Bakteriöl.*, I. 1896, xix. 97, 127.

<sup>2</sup> *Amer. Med.*, Aug. 15, 1903, p. 272.

<sup>3</sup> *Centralbl. f. Bakteriöl.*, I. Orig., 1904, xxxv. 605.

fatality; uninoculated, 10,000, 13 attacks and 75 per cent. fatality.

### PASSIVE IMMUNITY TO CHOLERA

The protection conferred by Haffkine's prophylactic is of the nature of active immunity, the blood of the patient gaining bactericidal power, and thus destroying those organisms which first enter the body and tend to cause infection. It has already been mentioned that the prospects of obtaining a bactericidal or antitoxic serum as a cure for the disease are not hopeful. Popoff<sup>1</sup> vaccinated a cow with cholera vibrios, and found that the milk contained a protective substance which conferred some degree of immunity on guineapigs. This substance was destroyed by boiling the milk.

A serum prepared by Schurupow has been used in Russia in cases occurring in children, 100–180 c.c. of serum being given, along with 100 c.c. of warm saline solution for each year of the child's life. The serum must be given early, within twelve hours of the onset of the disease.<sup>2</sup>

### CONCLUSIONS

1. Prophylactic inoculation with Haffkine's or Kolle's vaccine confers a certain measure of protection against cholera, and should be employed by those who are called upon to reside in districts in which they will be exposed to infection. It should probably not be performed in the actual presence of an epidemic, owing to the increased susceptibility induced during the first few days after the injection.

2. At present no therapeutic serum has been adequately proved to possess curative properties, and in the treatment of the disease Rogers's saline infusions should be employed along with other therapeutic measures.

<sup>1</sup> *Rousski Vra'ch*, 1893, No. 10.

<sup>2</sup> *Kiibanskaja, ibid.*, 1910, p. 8.

## CHAPTER XIII

### ENTERIC FEVER

**Causal organism.**—The *Bacillus typhosus* was discovered by Eberth in 1881, and is consequently often called Eberth's bacillus. It is found in considerable quantities in the alimentary canal of infected persons, but also exists in large numbers during the first week or so of the disease in the blood of the infected individual; later it is found in the spleen, in the lymphatic glands of the abdomen, and in the gall-bladder, where it sometimes forms the nucleus of a gall-stone. The organisms are excreted to a certain extent in the urine, as well as in the fæces, and appear in the sputum of cases complicated by lesions of the lungs or larynx. It appears, therefore, that the disease cannot be considered a local infection only, but is of the nature of a septicæmia or general infection (Wright).

**Complications.**—As in other infective diseases, the complications met with in the course of enteric fever, or during convalescence from it, are largely due to secondary invasion by other organisms which effect a lodgment in tissues worn out by conflict with a primary illness. The hectic temperature met with in the fourth week in severe cases of enteric fever is probably due to the action of pyogenic bacteria; while to these, or, in some instances, to the *Bacillus coli*, are usually to be ascribed most of the suppurative lesions (periostitis, perichondritis, otitis, epididymitis, etc.) which are seen in the later weeks. Venous thrombosis, so often met with in convalescence, is also to be attributed to pyogenic bacteria. In some cases, however, typhoid bacilli are found in local suppurative lesions,

but although they are occasionally the primary cause, they are usually implanted in the favourable soil of lesions caused by other bacteria. Possibly the *B. typhosus*, when its virulence is somewhat reduced, becomes a pyogenic organism, as Donzello<sup>1</sup> maintains. The cystitis which sometimes occurs, though it is rarely met with apart from catheterization, may be due to the bacilli contained in the urine; but the simple bacilluria which is frequently met with usually exists without any obvious cystitis.

**Toxins of *B. typhosus*.**—Cultures of typhoid bacilli do not as a rule contain any considerable quantity of free toxic matter, but the bodies of dead bacteria are themselves poisonous. Hence the toxins of the *B. typhosus* are generally spoken of as "intracellular." They are very unstable bodies.<sup>2</sup> Chantemesse claims to have succeeded in growing the bacilli in a special medium containing spleen-pulp and bone-marrow, and from this to have obtained a toxin of considerable potency, which he has used for the preparation of an antitoxin.

Aronson<sup>3</sup> also devised a medium in which toxins were formed, but was unsuccessful in preparing an antitoxic serum. Meycr and Bergell<sup>4</sup> were rather more successful with the toxins of bacilli grown in a peptone-broth prepared from the spleens of oxen, and regard the prospect of a curative use of their serum as hopeful.

## DIAGNOSIS

**Agglutination.**—The method of performing this diagnostic test has been fully dealt with in an earlier chapter on serum diagnosis in general (p. 77), to which reference should be made, so that it is only necessary now to point out the fallacies which surround the test when applied to typhoid infections.

<sup>1</sup> *Lo Sperimentale*, 1901, lv. 670.

<sup>2</sup> Macfadyen, *Brit. Med. Journ.*, 1906, i. 905.

<sup>3</sup> *Berlin. klin. Woch.*, 1907, p. 572.

<sup>4</sup> *Ibid.*, May 6,

**Sources of error.**—(1) Apart from the possibility of error due to a spurious appearance of agglutination, it must be borne in mind that a certain number of normal individuals, who have never suffered from enteric fever, possess a serum with some clumping power over typhoid bacilli, while not all patients suffering from the fever present the reaction. Thus Lobiesen<sup>1</sup> found that, out of 350 cases which were clinically enteric fever, 328 reacted positively to the Widal test with a dilution of 1 : 50 ; 17 agglutinated in a dilution of 1 : 10 or 1 : 25 ; two cases reacted only at 1 : 5 ; and two failed to react at all. The great majority of the patients (289) gave a positive reaction within the first two days after admission. Of 151 patients suffering from diseases other than enteric fever, in whom there was yet a suspicion that the malady might be of this nature, four reacted positively in dilution of 1 : 25, two at 1 : 10, and 123 were negative at this dilution. Of the first four, three were proved by necropsy to be suffering respectively from acute tuberculosis, retroperitoneal abscess, and calculous pyelitis with tubercular meningitis. Of 61 healthy persons who had not had enteric fever, one reacted positively in dilution of 1 : 25, and eight at 1 : 10. Lobiesen believes that a positive reaction in a dilution of 1 : 50 is pathognomonic of enteric fever, but that absence of the agglutination does not exclude the existence of the disease. Blanchi,<sup>2</sup> among 167 patients with enteric fever, found three cases which did not react to the test. The diagnosis was confirmed by necropsy.

Rolleston<sup>3</sup> in South Africa found that the test was positive in only 64·5 per cent. of his cases, and thinks that the low percentage may be accounted for by some difference in the strain of bacilli used for the test-cultures, as compared with the organisms which had infected the patients. Kohler<sup>4</sup>

<sup>1</sup> *Zeit. f. klin. Med.*, Bd. xliii., Hft. 1 and 2.

<sup>2</sup> *Giornale Medico del Regio Esercito*, 1901, No. 5.

<sup>3</sup> *Brit. Med. Journ.*, 1901, ii. 1084.

<sup>4</sup> *Münch. med. Woch.*, 1903, p. 1379.



found that among 100 patients suffering from diseases other than enterica 12 gave a reaction in dilutions of 1:20. Among these 12, one agglutinated at 1:50, two at 1:40, and three at 1:30. Hence it appears that even an agglutination reaction in a dilution of 1:50 is not a definite proof of the existence of enteric fever.

(2) Again, it has been frequently noticed, and the present writers have had many opportunities of observing, that in some severe and fatal cases of enteric fever, in which the diagnosis is subsequently confirmed by post-mortem examination, no agglutinative power is found throughout the illness. The absence of agglutination is parallel to the absence of resistance exhibited by the patients towards the infective agent.

(3) The clumping power of the serum is not developed at the beginning of the attack. The exact period at which it may be looked for is not certain, but many observations have shown that during the first week or even ten days an absence of agglutinative power is rather the rule than the exception. Brion and Kayzer,<sup>1</sup> however, find the reaction positive in the first week in 50 per cent. of all cases.

(4) The serum of persons who have been inoculated against typhoid fever (p. 246) will contain agglutinins for a considerable time after this procedure; the test as applied to a "chance" sample of serum is therefore useless in such patients as a criterion of a suspected attack of enteric fever.

(5) Certain other infective and general diseases are apparently capable of producing substances in the serum which will agglutinate typhoid bacilli. Infection with *B. coli communis* seems to produce this effect in some cases.<sup>2</sup> Allusion has already been made to the experiments of Posselt and Sagasser,<sup>3</sup> who found increased clumping power towards *B. typhosus* in cases of dysentery.

<sup>1</sup> *Deut. Arch. f. klin. Med.*, lxxxv., Hft. 5 and 6.

<sup>2</sup> Cf. Lubowski and Steinberg, *ibid.*, 1904, p. 396 (proteus-infection, staphylococci, etc.).

<sup>3</sup> See p. 81.

Morgan<sup>1</sup> found that of six cases of cerebro-spinal meningitis, three gave a "Widal" reaction when the serum was diluted 1 : 50, and one produced a definite but less marked agglutination.

The effect of jaundice in causing agglutination was first pointed out by Grünbaum,<sup>2</sup> and has been confirmed by other observers. The reaction does not seem to be manifested in all cases of jaundice, but it may occur with sufficient frequency to cause us to regard the test as unreliable when this condition is present. Bile itself does not appear to agglutinate the bacilli in all cases, but in certain individuals and conditions of health it may produce the reaction. The exact body which is active in this way is not known, but its effect is analogous to the action of formalin and other chemical substances. It must, however, be borne in mind that infection of the biliary passages with *B. typhosus* is not uncommon, and that the jaundice may be a result of this—the occurrence of the reaction thus being the "exception that proves the rule."

(6) All strains of *B. typhosus* are not agglutinated with equal facility. It is therefore necessary to make use of a culture in which the bacilli have been proved to possess this faculty. Klein finds that culture of the bacilli on gelatin induces greater agglutinative properties, and advises the use of such cultures emulsified with salt-solution.

(7) Kraus<sup>3</sup> finds that the effect of pneumonia is to inhibit the agglutinative power. This may be demonstrated by adding the serum of a pneumonic patient to that of one suffering from enteric fever. The coexistence of pneumonia with enteric fever would, therefore, theoretically prevent the appearance of Widal's reaction, and the test would be useless as a means of differentiating the two diseases. These

<sup>1</sup> "An Account of an Outbreak of Spotted Fever, etc." Swansea 1909.

<sup>2</sup> *Münch. med. Woch.*, 1897, No. 13.

<sup>3</sup> *Zeit. f. Heilkrankh.*, Bd. xxi., Heft 5.

observations, however, need further confirmation. Kissel and Mann,<sup>1</sup> on the other hand, found that two cases of croupous pneumonia gave the reaction, though they were not suffering from enteric fever.

**Value of the test.**—On the whole it may be said that the reaction is not infallible, but has a margin of error of perhaps 5 per cent. Abbott<sup>2</sup> studied 4,154 cases, and found the error only 2·8 per cent. We believe that, with a time-limit of thirty minutes, a positive reaction in a dilution of 1 : 20 implies either the existence of enteric fever or a past attack within recent years; a reaction in a dilution of 1 : 50 implies either existing disease or a very recent attack; and a reaction in a dilution of 1 : 200 is positive proof of present typhoid infection.

The test is said to be of special value in the case of children. Gershel,<sup>3</sup> among 84 cases of enteric fever in infants, found the reaction positive in 81, while it was negative in 115 patients who were suffering from other diseases. Other writers also agree with this estimate of the use of the test in children.<sup>4</sup>

It is generally stated that the agglutination reaction is of no use as a means of *prognosis*. This is probably true as far as the rapidity and completeness of the clumping are concerned; but there is reason to think that a case which, clinically, is almost certainly a severe attack of enteric fever, but which gives no reaction, is likely to end fatally, as the absence of agglutinative power in such instances seems to be associated with an absence of resistance.

**Persistence of the reaction.**—H. French and Louisson<sup>5</sup> find that as a rule the reaction disappears rapidly after the patient's recovery, but that in exceptional cases (7·5 per cent.) it may remain for years (eight

<sup>1</sup> *Münch. med. Woch.*, May 2, 1899.

<sup>2</sup> *Philadelphia Med. Journ.*, Feb. 25, 1899.

<sup>3</sup> *Med. Record*, Nov. 26, 1901.

<sup>4</sup> Josias and Tollemer, *Med. Press and Circ.*, Aug. 26, 1903, p. 217.

<sup>5</sup> *Guy's Hosp. Repts.*, 1907, lxi, 235.

years and probably longer). Gaehtgens<sup>1</sup> found the reaction positive in one case thirty-five years after an attack.

**Mandelbaum's reaction.**<sup>2</sup>—This modification of the agglutination reaction consists in growing the bacilli in bouillon along with a little immune serum. The organisms then form chains and clumps, whereas if the serum used is not immune they develop as usual. This reaction may be used as a test for the disease. Ast<sup>3</sup> considers it useful for the discovery of "carriers," but admits that an incomplete reaction may occur with the serum of normal persons.

**Ophthalmic reaction.**—Chantemesse<sup>4</sup> suggests as a test the use of a powder derived from the bacilli, which is applied to the conjunctiva and causes inflammatory reaction in patients suffering from enteric fever, just as tuberculin does in tuberculous subjects.

This test has been tried by Kraus, who finds that 60 per cent. of patients with enteric fever react positively. Cohn and Entz find that healthy persons may also give a reaction.<sup>5</sup>

Meroni holds that a negative result after twenty-four hours excludes enteric fever, while a positive reaction raises a probability, but not a certainty, of typhoid infection.<sup>6</sup>

## SERUM TREATMENT

**Chantemesse's serum.**—By means of the toxins prepared as already mentioned (p. 230), Chantemesse<sup>7</sup> has produced a serum for the cure of enteric fever. It is prepared by inoculation of horses with the toxin in the usual manner. The process of inoculation is long and tedious, as very small doses must be employed at first; otherwise the

<sup>1</sup> See *Lancet*, 1907, i. 1363.

<sup>2</sup> *Münch. med. Woch.*, 1910, p. 855.

<sup>3</sup> *Ibid.*, p. 2634.

<sup>4</sup> *Deut. med. Woch.*, 1907, p. 1572.

<sup>5</sup> See Wolff Eisner, *op. cit.*, sub. Tuberculin.

<sup>6</sup> *Münch. med. Woch.*, 1908, No. 26.

<sup>7</sup> *La Presse Méd.*, 1901, No. 93, p. 285.

horses may be killed by the toxin—Chantemesse speaks of losing several in the course of his investigations. The immunization of the animals was begun in 1896, whereas the first experiments with the serum on patients suffering from enteric fever were apparently carried out in 1900 (?). It may be concluded that at least two years were consumed in the preparation of the remedy. The results of this method of treatment, as recorded by its author, are very encouraging. It is difficult to ascertain the average mortality of the disease, as it varies much in severity in different years, the reasons for this variability not being known. In Paris, in the years 1899 and 1900, the death-rate was 18·5 per cent. ; in 1901, from January to October, it was 29 per cent. among 371 patients treated in nine hospitals. Chantemesse treated 100 patients by his method, with six deaths. All those who were treated before the tenth day recovered. Of those treated later, three cases died of perforation of the intestine ; one (injected on the twenty-first day of the illness) of pneumonia ; one (injected on the twenty-fifth day) of hyperpyrexia, and one of a sacral bed sore acquired before admission to hospital. Two subsequent cases, which were injected on admission to hospital in a moribund condition, are not included in the statistics.

According to some more recent statistics,<sup>1</sup> while the mortality in fourteen hospitals where the serum was not used amounted to 17·3 per cent. (3,595 cases, with 763 deaths), in the Bastion Hospital, where the serum was used, the mortality was only 3·7 per cent. (712 cases, 27 deaths).

Chantemesse gives charts of some of the cases treated by the serum, showing that the injections are followed by a rapid fall of temperature and improvement in the pulse. The earlier the serum is administered, the more marked is the effect. If the remedy be given before the eighth day in cases of ordinary severity, the disease may be cut short within a period of a few days. Sometimes the first improve-

<sup>1</sup> Chantemesse, *Paris Med. Journ.*, 1906, i, 3.

ment is not maintained, and the temperature rises again on a later day ; in such instances a second injection should be given, and may be followed by rapid recovery. The accompanying charts, modified from those given in Chantemesse's article, show the results obtained in some of his cases (Charts 1 and 2).

Besides the effects on the pulse and temperature, the serum has a beneficial influence on the excretion of urine,

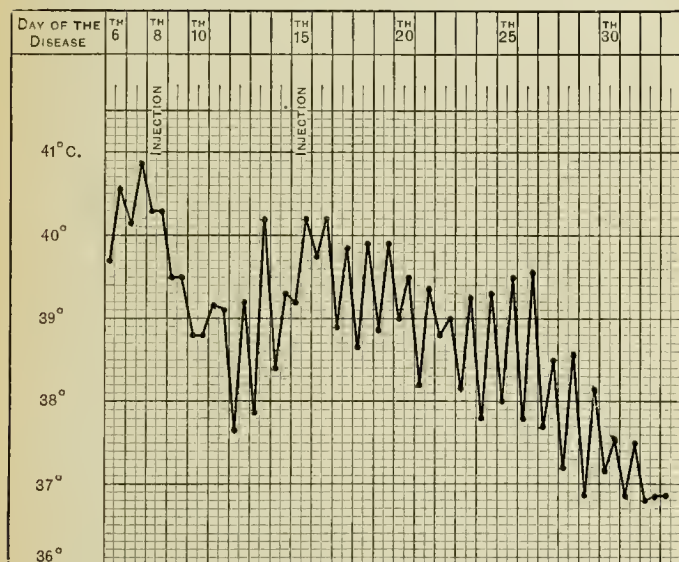


CHART 1.—ILLUSTRATING THE EFFECTS OF CHANTEMESSE'S SERUM.

which increases in quantity as the pulse and temperature fall. Albuminuria is not caused by the serum itself—a point in which it appears to differ from diphtherial anti-toxin, which is accused of causing the appearance of albumin—indeed, in cases in which there is already albuminuria, this may decrease as the result of serum treatment. A hyperleucocytosis is produced in the blood, in opposition to the leucopenia (defective number

of leucocytes) which is characteristic of enterica. The leucocytosis is exactly similar to that which is normally

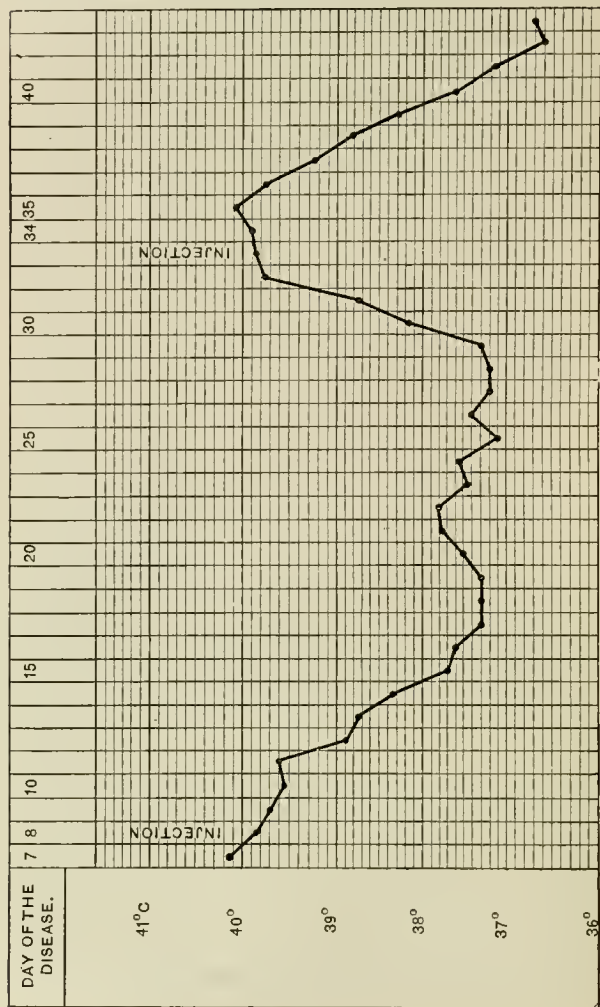


CHART 2.—ILLUSTRATING THE EFFECTS OF CHANTEMESSE'S SERUM.



seen in convalescents from enteric fever. The myelocytes which are present during the disease disappear, while the other varieties of cells (lymphocytes, eosinophile cells, and multinucleate leucocytes) increase to their normal amount : they may even be in excess at first.

Complications are rare in cases treated with serum, but are not entirely absent. Chantemesse noted in his first series one case of perforation and one of pneumonia, both fatal ; and others of otitis media, hæmorrhage, and phlebitis, all of which recovered. Probably the serum has no direct influence on the occurrence of complications ; these are due to other organisms, which are unaffected by it ; but if it control and cut short the typhoid infection, it must indirectly diminish the risk of secondary lesions.

The serum is injected under the skin of the forearm in the neighbourhood of the usual point for venesection. The skin and the syringe are carefully sterilized, and the veins of the part must not be wounded. The ordinary dose of serum is 10 or 12 c.c. A second dose may be given at the end of eight or ten days, if the temperature has risen again, or if there is any other indication ; the second dose may be smaller than the first—4 or 5 c.c. There are two indications for giving a smaller dose than the above-mentioned as a first injection, viz. (1) when the patient comes under treatment in quite the early days of the disease (fifth or sixth) ; and (2) when the disease has already lasted for a considerable time, and the general intoxication is profound. In these cases 5 to 8 c.c. are sufficient.

The injections are followed by a reaction, shown by a rise of temperature, which quickly falls again. Chantemesse attributes this reaction to the great destruction of the bacilli which is induced by the serum, and to the consequent absorption of a large dose of their toxins. He considers his serum to be bactericidal as well as antitoxic. It is difficult to accept this explanation in view of the nature

of the serum, which, from its manner of preparation, should be purely antitoxic, and not bactericidal. An additional argument against Chantemesse's view may be derived from the fact that the injection of this serum does not increase the agglutinative power of the blood.<sup>1</sup>

Along with the serum, other treatment should not be omitted, especially reduction of temperature by baths, and the plentiful supply of liquid nourishment to the patient. It may be necessary to stop milk-feeding for a time after the injection, as milk often appears to be ill digested; it may be resumed again as the temperature falls.

No bad effects are produced by the serum, with the exception of slight erythema, which only appeared in two of Chantemesse's 100 cases.

Besides Chantemesse himself, Bontleux<sup>2</sup> treated 15 cases with the serum; all of these recovered. Josias<sup>3</sup> reports 50 cases in children in which he used the remedy. Among them there were two deaths, a mortality of 4 per cent. Simultaneously, in other children's hospitals in Paris the mortality was 14·2 per cent. He confirms the benefit derived from early administration of the serum, and states that, though relapses occurred in four cases, they were mild in type. The doses given amounted to 1 c.c. for each 30 kilogrammes of body-weight. In a certain proportion of cases in young children pain in the bowels was complained of, some days after the injections; and this was at times so severe as to create a suspicion of peritonitis. No ill effects, however, actually ensued. Osteitis and periostitis occurring during convalescence from enteric fever are said to be benefited by the serum.

Before we can pronounce a definite verdict on the value

<sup>1</sup> Josias and Tollemer, Congress of Madrid, 1903. *La Presse Méd.*, June 24, 1903, p. 468.

<sup>2</sup> Quoted by Chantemesse, *loc. cit.*

<sup>3</sup> International Medical Congress of Madrid. See *Med. Press and Circular*, July 29, 1903, p. 109; *Ann. de Méd. et Chir. Infantiles*, 1903, No. 11, p. 367.

of this serum it will be necessary to wait until a larger number of physicians have used it in the treatment of enteric fever and recorded their results. Up to the present day, this preparation does not seem to have been extensively used in treatment. Chantemesse's results, however, are very encouraging, although 712 cases form far too small a material on which to base an opinion, since the disease is so variable in its severity at different times and different places.

**Other serums.**—W. V. Shaw<sup>1</sup> obtained toxic material by "digesting" *B. typhosus* in normal blood-serum. He injected this into a horse and obtained a serum which had some protective power, while the injections were at first followed by a fall in the bactericidal property of the horse's serum—"negative phase." From this it would appear that the serum is antibacterial rather than antitoxic, and hence comparable with that mentioned in the following section rather than with Chantemesse's preparation.

Macfadyen immunized a horse by injecting it with endotoxin, prepared by grinding up typhoid bacilli at the temperature of liquid air, and showed that the serum was both antitoxic and bactericidal. Hewlett<sup>2</sup> continued the observations and employed the serum in 9 cases of enteric fever; he concluded that 8 patients were benefited and that in 2 of these the course of the fever was decidedly shortened. Goodall<sup>3</sup> tried it in 26, and Bruce<sup>4</sup> in 5 cases; benefit being observed in 9 of these last 31 cases—not a very striking achievement.

Rodet and Lagriffoul<sup>5</sup> state that by injecting living bacilli they get an antitoxic serum which they used with advantage in 27 cases of enteric fever. To be of use the remedy must be given before the eleventh day.

<sup>1</sup> *Lancet*, 1903, ii. 948.

<sup>2</sup> *Proc. Royal Soc. Med.*, 1909, ii., Medical Section, p. 245.

<sup>3</sup> *Op. cit.*, p. 254.

<sup>4</sup> *Op. cit.*, p. 262.

<sup>5</sup> *Compt. Rend. Soc. de Biol.*, 1910, lxxviii. 604.

Kraus and Stenitzer<sup>1</sup> prepared an anti-endotoxic serum which proved beneficial in doses of 30-40 c.c. They also insist on administration early in the disease.

#### BACTERICIDAL SERUM

**Antityphoid serum.**—The serum of convalescents from enteric fever is bactericidal and not antitoxic in its action; and most of the serums on the market, which are professedly “antityphoid,” are of similar nature. They are prepared by immunizing a horse with the actual bacilli of enteric fever. Chantemesse<sup>2</sup> alludes to experiments made by Widal and himself in order to produce a serum of this nature, but he speaks of the results obtained as unsatisfactory. In 1898 Bokenham<sup>3</sup> prepared an antityphoid serum by inoculating a horse with filtered cultures of the bacilli and then with the dead bodies of the organisms themselves; he found that the serum acted as a protective to rabbits.

Krumbein<sup>4</sup> used for inoculation, first, filtered cultures, then bacteria killed by carbolic acid. The bacilli were grown for fourteen days in broth, to which 0·5 per cent. of phenol had been added. The cultures were injected subcutaneously. Considerable constitutional disturbance may be produced, and abscesses may also form at the seat of injection. After a point had been reached at which 150 c.c. were given for a dose, the serum of the horse was drawn off and used. In subsequent experiments the living bacilli were injected during the later periods of the immunizing process.

It appears that the typhoid bacillus is modified to some extent by its surroundings, and that different strains of bacteria thus produced, taken from different cases, may act variably towards a particular serum. In other words, a

<sup>1</sup> *Deut. med. Woch*, 1911, p. 577.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *Trans. Path. Soc. Lond.*, 1898, p. 373.

<sup>4</sup> Ainley Walker, *Journ. of Pathol. and Bacteriol.*, 1901. vii. 251.

serum is found to have a more marked effect on the strain of bacilli from which it was prepared. It is, therefore, advisable to make use of several strains in the immunization of the horse. The serum of the horse becomes highly agglutinative towards the bacilli as the result of the treatment. Walker considers that the agglutinative power increases practically *pari passu* with the protective property, but that the two are not directly proportional to one another. He finds that the serum prepared as above is antitoxic as well as antibacterial. He also makes the suggestion that the horse should be immunized against the *B. coli communis* as well as against the *B. typhosus*, or that some "anti-coli" serum should be added to the antityphoid serum for therapeutic use. Rodet and Lagriffoul<sup>1</sup> also state that after the injection of living bacilli the serum obtained is antitoxic. They advise the use of such serum, which should be administered before the eleventh day of the disease.

Experience at the present day is not very favourable to the use of an antibacterial serum in the treatment of enteric fever. The present writers have seen the ordinary serum which is on the market tried in several cases of the disease, but in none of them was it possible to be sure of any definite benefit accruing to the patient. Relapse was not prevented by the use of the serum. Walker concludes that "most antityphoid sera which have been prepared have given no marked assistance in the treatment of the disease in man." Reasons for this have been already suggested. Moreover, in the later stages of the disease the bacilli are for the most part localized in the alimentary canal, and the toxins are probably absorbed, as in cholera, without any further escape of the organisms into the general circulation. If so, it may be difficult for the antibacterial serum to reach them; and attention should be turned to the preparation rather of an antitoxic serum than of one that is germicidal. In the second place, it may be that the copula or immune

<sup>1</sup> *Compt. Rend. Soc. de Biol.*, 1910, lxviii, 604.

body present in horse-serum is not capable of uniting with the alexine or complement found in human blood, in which case no bacteriolysis would be produced. It has further to be remembered that enteric fever is characterized by a gradual onset, so that it is seldom recognized until it has lasted at least five or six days. Hence the first requisite in the administration of any kind of serum—early injection—is generally impossible, and it is unreasonable to expect as good results to occur as can be obtained in diphtheria. It is possible that by the time the serum is used there may be a deficiency of complement in the patient's blood, and that bacteriolysis may not occur, even if the copula supplied be suitable. Finally, if the destruction of the bacilli is brought about by the interaction of the serum with the leucocytes of the patient, the leucopenia characteristic of the disease may militate against the efficacy of the remedy.

#### ANTITYPHOID EXTRACT OF JEZ

Jez<sup>1</sup> starts with the assumption that the serum obtained from immunized animals is bactericidal and not antitoxic, and that such a serum is of no value for the treatment of enteric fever. Some other method of conferring immunity must be tried. Now, Wassermann found that the spleen, bone-marrow, and lymphatic glands of an immunized animal had protective properties; and Jez has made use of this discovery to prepare a substance which he considers to be curative of enteric fever. He makes his antityphoid extract by rubbing up in a mortar the brain, spinal cord, spleen, marrow, etc., of immunized rabbits, and adding to the pulp thus obtained saline solution, to make an emulsion, along with a small amount of alcohol and of carbolic acid. The fluid is filtered after it has stood for a time, to ensure solution of the protective bodies. In later experiments Jez added also a certain proportion of pepsin, presumably in order to facilitate solution.

The filtered fluid is antitoxic, but not agglutinative or

<sup>1</sup> *Wien. med. Woch.*, Feb. 18, 1899, p. 346

bacteriolytic. As a remedy for enteric fever, it is given by the mouth; but if for any reason this is impossible, it can be administered subcutaneously. A tablespoonful constitutes a dose, which may be given every two hours, or more frequently. Considerable quantities are needed for each case, reaching a pint or more.

Jez finds that, as the result of treatment with his extract, the temperature falls, the pulse improves, and the general condition of the patient is ameliorated. Diarrhœa is usually checked. Sometimes sweating is produced by the action of the remedy. Jez records the trial of the extract in eighteen cases, all of which recovered.

These results are confirmed by Kluk-Kluczycki,<sup>1</sup> who finds that the fever-reducing effect is manifested within twenty-four hours, an apyrexial condition being often reached within three weeks—which may also occur even in untreated cases. Eichhorst<sup>2</sup> has also tried the extract in a small number of cases (12), and is favourably impressed with the results produced; and a similar verdict is pronounced by du Mesnil de Rochemont<sup>3</sup> and by Einhorn,<sup>4</sup> who observed a reduction of fever and some mental improvement. On the other hand, Pometta<sup>5</sup> found Jez's preparation quite useless.

The use of Jez's extract does not seem to have become at all general, so that there is not sufficient information available upon the subject to enable us to form a satisfactory judgment as to its efficacy. The idea underlying it is not to be neglected, as Wassermann's experiments, confirmed by Jez, seem to point to the existence of a protective principle in the organs of immunized animals (*cf.* p. 212, Plague). This does not, however, necessarily imply its value as a cure for the disease.

<sup>1</sup> *Wien. klin. Woch.*, 1901, No. 4, p. 84.

<sup>2</sup> *Therap. Monatsh.*, 1900, p. 115.

<sup>3</sup> *Ibid.*, Jan., 1904, p. 13 (7 cases).

<sup>4</sup> *Med. Record*, Jan. 16, 1904, p. 81 (3 cases).

<sup>5</sup> *Wien. med. Woch.*, 1901, No. 46.



## ANTITYPHOID INOCULATION

**Wright's vaccine.**—Experiments were made by Pfeiffer and Kolle<sup>1</sup> in 1896 as to the effect of inoculating patients with cultures of typhoid bacilli; but although it was shown that the blood of those so treated had a protective influence on guineapigs, no practical use seems to have been made of the method. It is to Wright that the practical introduction of vaccination as a means of prophylaxis against enteric fever is entirely due.

The vaccine originally used by Wright<sup>2</sup> consisted of cultures of *B. typhosus* in broth, grown for four weeks, and then sterilized by heating for ten to fifteen minutes at 60° C. For this vaccine a large number of separate cultures are mixed together, so as to obtain a fluid of the standard strength. Special flasks also are used for the preparation of the cultures, in order to facilitate the subsequent mixing. The bacterial content of the material can be roughly gauged by its opacity to light, for the measurement of which Wright devised an ingenious arrangement. A small amount of carbolic acid or lysol is subsequently added to ensure sterility and the preservation of the vaccine.

The dose used for an injection in man was the minimal lethal dose for a guineapig weighing 100 grm., or rather the proportional fraction of the dose which proves fatal to one of the ordinary weight (250 to 300 grm.). A virulent culture will contain the requisite quantity in 0·5 c.c., but with weaker vaccine it is necessary sometimes to give as much as 1·5 c.c. Wright also used a vaccine consisting of agar-cultures of the bacilli grown for twenty-four hours and sterilized at 60° C., a modification still preferred by many workers as being less toxic than the broth-vaccines.

The injections are followed by redness and pain at the

<sup>1</sup> *Deut. med. Wch.*, 1896.

<sup>2</sup> *Lancet*, 1901, i, 150.

site of inoculation, with some lymphangitis and enlargement of neighbouring glands. There may be nausea and even vomiting, and there is considerable feeling of illness, with some rise of temperature. Occasionally in a neurotic patient a condition approaching collapse is observed. These symptoms pass off rapidly without leaving any permanent ill effects, but they are severe enough to act as a very real deterrent.

Wright found, later, that better results were obtained if the vaccine was prepared from twenty-four-hour cultures grown upon the surface of agar, and, after emulsification, standardized to contain 1,000 millions of typhoid bacilli in every cubic centimetre. Sterilization is effected by heating for half an hour at  $56^{\circ}$ – $58^{\circ}$  C., and 0.5 per cent. lysol is then added. The dose is 500 or 1,000 millions of bacilli. Two injections, the first of 500 and the second (fourteen days later) of 1,000 millions, are, however, preferable. We frequently give *three* doses, the first of 500 millions, the second of 1,000 millions after an interval of ten days, and the third of 1,000 or 2,000 millions eight days after the second. After such injections the local reaction and neighbouring lymphangitis are the only resulting inconveniences.

The immediate result of the vaccination is to produce a lowering of the resistance offered by the individual to infection by enteric bacilli. If large doses of the vaccine are given, this fall in immunity may be very marked, and may last for some weeks. If small doses are given, the fall in resistance is very slight and transitory. For these reasons it is advisable to make use of small doses, repeated if necessary, rather than one large dose. It is also important not to vaccinate in the presence of an epidemic, as such a procedure would tend to make the subject more liable to contract the infection.

Almost all the statistics as to the efficacy of Wright's vaccination are derived from observations on different units of the British army in South Africa during the

Boer War, and in India, Egypt, and Malta. On the following page are some of the figures given by Wright himself.

Cayley<sup>1</sup> also gives favourable figures with regard to the use of inoculation in the members of the Scottish National Red Cross Hospital. Among 57 inoculated persons in the 1st Section no attacks occurred; among 82 of the 2nd Section, the greater number were inoculated with old vaccine, and five orderlies developed enteric fever; one nurse refused inoculation, and she also suffered. Among the 3rd Section (20) all were inoculated, and no cases of the disease occurred. Cayley considers that cases which do occur in inoculated persons are milder and run a shorter course than in the uninoculated.

Birt<sup>2</sup> quotes his experience in an epidemic at Harri-smith. Among 947 unvaccinated patients the mortality was 14.25 per cent., while of 263 who had been inoculated the death-rate was only 6.8 per cent. These figures point to the disease being of a milder character in those who have been vaccinated. Kuhn<sup>3</sup> used three inoculations, and found that no "negative phase" followed the third. He records good results, and states that immunity lasts for one year. Bessau<sup>4</sup> and Vincent<sup>5</sup> also assert that there is no period of increased susceptibility (negative phase) after the use of the vaccine.

In the United States army the procedure consists in giving three doses of 300 millions into the deltoid muscle (Fletcher).<sup>6</sup> Inoculation is compulsory on all recruits. Statistics show that whereas between 1901 and

<sup>1</sup> *Brit. Med. Journ.*, 1901, i. 84.

<sup>2</sup> *Ibid.*, 1902, i. 75. Cf. Ward, *Journ. R.A.M.C.*, 1906, vi. 431.

<sup>3</sup> *Abstr. Centralbl. f. Bakt.*, I. Ref., 1907, xl. 602. Cf. Eichholz, *Münch. med. Woch.*, 1907, p. 777.

<sup>4</sup> *Berl. klin. Woch.*, 1912, No. 47.

<sup>5</sup> *Compt. Rend. Acad. Sci.*, 1912, clv. 784.

<sup>6</sup> *Journ. Amer. Med. Assoc.*, April 8, 1911. Cf. Phallen and Callison, *Med. Record*, 1911, lxxx. 1203.

# STATISTICS OF ANTITYPHOID INOCULATION <sup>1</sup>

Group	Total numbers	Cases of enteric fever	Percentage incidence	Deaths	Percentage death-rate	Case-mortality
British Army in South Africa ... { ... {	Inoculated ... 4,502 Uninoculated ... 25,851	44 657	0·98 2·54	9 146	0·2 0·56	1 in 4·9 1 in 4·5
15th Hussars ... { ... {	Inoculated ... 360 Uninoculated ... 179	2 11	0·55 6·14	1 6	0·27 3·25	1 in 2 1 in 2·2
Garrison of Ladysmith ... { ... {	Inoculated ... 1,705 Uninoculated ... 10,529	35 1,489	2·05 14·14	8 329	0·47 3·12	1 in 4·7 1 in 4·5
Garrison of Egypt and Malta ... { ... {	Inoculated ... 720 Uninoculated ... 2,669	1 68	0·14 2·55	1 10	0·14 0·37	1 in 1 1 in 6·8
British Army in India, 1900 ... { ... {	Inoculated ... 5,999 Uninoculated ... 54,554	52 731	0·87 1·69	8 224	0·13 0·58	1 in 6·5 1 in 3·3
British Army in India, 1901 ... { ... {	Inoculated ... 4,883 Uninoculated ... 55,955	32 744	0·66 1·33	3 199	0·06 0·36	— —
City Imperial Volunteers { ... {	Inoculated ... 700 Uninoculated ... 494	60 39	8·5 7·9	9 11	1·3 2·2	1 in 6·7 1 in 3·5

<sup>1</sup> A. E. Wright, *The Practitioner*, March, 1904, p. 370. The groups consisting of the largest numbers are here selected. For complete statistics reference may be made to the original article.

1908 the incidence of the disease varied between 3·20 and 6·99, with a mortality of 0·27–0·88, in 1912, when vaccination was extensively practised, the figures were 0·31 morbidity and 0·044 mortality (Revoul).<sup>1</sup>

Fox,<sup>2</sup> in India, uses two inoculations of 500 and 1,000 millions respectively, given at an interval of not more than ten days. The vaccine, consisting of bacilli killed at a temperature of 53° C., should be kept for three weeks before use, but not employed when it is more than three months old. Vaccination should be repeated after two years.

Bassenge and Mayer<sup>3</sup> suggest for purposes of vaccination the use of a clear fluid prepared according to Brieger's method by shaking typhoid bacilli with distilled water and filtering. The preparation is very stable, but immunity only lasts about three months.

Besredka,<sup>4</sup> who employs living typhoid bacilli sensitized by the addition of typhoid amboceptor from immune serum, having inoculated more than 15,000 individuals during a period of two and a half years, states that this preparation not only produces greater immunity, but also causes less local and constitutional effect than the "killed" vaccine.

The experience gained during the present European War forms a striking testimony to the value of antityphoid vaccination which has been extensively practised in the British army. Although the exact figures of inoculated and uninoculated are not available, some battalions went to the front composed entirely of inoculated men. On January 10, 1916, in answer to a question in the House of Commons, the Under-Secretary of State for War gave the following figures in connection with the distribution of cases of typhoid which had occurred in the British forces

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1913, lxi, 666.

<sup>2</sup> *Journ. Trop. Med.*, 1910, xiii, 367.

<sup>3</sup> *Deut. med. Woch.*, May 4, 1905.

<sup>4</sup> *Ann. de l'Inst. Pasteur*, 1913, xviii, 607.

in the field (the total numbers of troops involved are not permitted to appear) :—

	CASES	DEATHS	MORTALITY
Uninoculated ... ..	571	115	Per cent. 20·14
Fully inoculated within two years (two doses) ... ..	369	22	5·96
Partially inoculated (one dose)	210	13	6·19

It must be remembered that one attack of enteric fever does not protect against a second, as was at one time supposed. Experience in the South African War has definitely proved that second attacks are not by any means rare, although they seem, as a rule, comparatively mild in degree. Hence vaccination cannot be expected to produce absolute immunity, but, as might be expected, a similar degree of protection in the inoculated as exists among those who have previously suffered from an attack of typhoid fever; consequently infection, when it does occur in the vaccinated, is for the most part mild in intensity and anomalous in character. There is some reason to think that the protection thus afforded is less marked in those who are past the age of 30;<sup>1</sup> but it is also probable that individuals become progressively less susceptible as they advance in life; hence the above advice applies chiefly to those below that age. The temporary inconvenience<sup>2</sup> caused by the injections cannot be held to constitute

<sup>1</sup> Crombie, *Lancet*, 1902, ii. 426.

<sup>2</sup> Occasionally the symptoms produced by the original vaccine were severe and even alarming, though no fatal results are recorded. Thus, Lindsay (*Lancet*, 1905, ii. 827) saw a case in which an acute illness ensued, lasting fourteen days; and we have seen a similar case, in which there was very high fever (103°–104° F.) for several days, with headache, sleeplessness, and distension of the abdomen, following the second of two injections which were given at an interval of seven days.

a sufficient disadvantage to counterbalance the protection gained.

We repeat that those who are actually in the midst of an epidemic of enteric fever should not be inoculated with an ordinary vaccine, owing to the increased liability to contract the disease which at first ensues as a result of the treatment, but that if a reliable "sensitized" vaccine is available we should advocate its use, as a fair degree of immunity is thereby secured practically immediately.

### VACCINE TREATMENT

Petrushky<sup>1</sup> made experiments with a preparation which he called *typhoïn*, consisting of dead bacilli. He reported good results in cases of uncomplicated enteric fever, if the remedy were given early in the course of the illness. It is not suitable for patients in whom the disease is advanced and in whom there is already a tendency to heart-failure or general intoxication. Small, gradually increasing doses are given, and the first injections are accompanied by some antityphoid serum, to prevent ill effects.

Semple<sup>2</sup> also reports favourably on the effects of vaccine treatment in this disease. He used doses of 15–30 millions of bacilli daily for four to six days, and controlled the results by opsonic estimations. He recommends the employment of cultures derived from the individual patient.

Pescarolo and Quadrone<sup>3</sup> have used a vaccine of living typhoid bacilli upon patients suffering from the disease, and noted good results—fall of temperature and rapid recovery. They made use of agar-cultures attenuated by heating to 45°–50° C. The inoculation is followed by shivering and rise of temperature, while some redness and swelling may appear at the site of injection.

Larger doses have been used by later workers. Thus Smallman<sup>4</sup> at Quetta gave doses of 300 to 350 millions

<sup>1</sup> *Deut. med. Woch.*, 1902, p. 212.      <sup>2</sup> *Lancet*, 1909, i. 1668.

<sup>3</sup> *Centralbl. f. inn. Med.*, 1908, No. 40, p. 989.

<sup>4</sup> *Journ. Royal Army Med. Corps*, 1909, xii. 136.



every third day; he reports a series of 36 cases with 3 deaths and no relapses. Callison,<sup>1</sup> who has collected extensive statistics on the subject, himself uses doses of 300 millions to start with, increasing by 100 millions each successive dose at intervals of three or four days. He treated 38 cases with a mortality of 5 per cent. and a relapse-rate of 1 per cent. Among 423 collected cases the mortality worked out at 5.4 per cent., and the relapse-rate at 6.5 per cent. If Osler's estimate of the general mortality of typhoid fever at 5-12 per cent. in private and 7-12 per cent. in hospital cases is correct, these figures point to some degree of benefit, but they are not large enough to be very impressive. Hollis<sup>2</sup> found some apparent clinical improvement as the result of vaccines, but believed that relapse is more frequent—a curious result of a method devoted to increasing the patient's resistance. Anders<sup>3</sup> states that vaccine treatment is dangerous in severe cases.

Gauchery<sup>4</sup> has collected 2,294 cases in which vaccines were used in enteric fever, and adds 25 of his own. He finds that 25 per cent. of all cases respond rapidly to the treatment; symptoms quickly ameliorate, but the temperature is less influenced, and an outburst of roseolar spots may occur. In 50 per cent. some benefit appears to result, but of a less striking nature; while 25 per cent. do not respond in any way. Taking all the cases together, the mortality is reduced from 9.9 to 5.7 per cent. Perforation of the intestine is rare in cases treated with vaccines, but there is no marked diminution in liability to hæmorrhage or in the number of relapses.

MacArthur<sup>5</sup> reported a series of 63 cases of typhoid

<sup>1</sup> *Med. Record*, 1911, p. 1129; *Amer. Journ. Med. Science*, 1912, clxiv. 350.

<sup>2</sup> *Med. Record*, 1910, lxxvii. 642.

<sup>3</sup> *Ibid.*, 1910, lxxviii. 1160.

<sup>4</sup> Thèse de Paris, 1914 (abstr. in *Journ. de Physiol. et de Path. Gén.*, 1914, xvi. 757).

<sup>5</sup> *Brit. Med. Journ.*, 1914, ii. 175.

fever treated with a vaccine prepared from the *B. typhosus*, which had been isolated in 58 cases from the patient's blood, and in 3 cases from the patient's fæces. With these autogenous vaccines there were 2 deaths (3·1 per cent.). The remaining cases were treated with a stock vaccine. The initial dose varied from 150–300 millions, and subsequently increasing doses were injected at intervals of two or three days. The disease was definitely rendered milder, and symptoms other than pyrexia were absent.

Vaccines have been recommended for the treatment of typhoid "carriers"—those who continue after an attack of the disease to harbour virulent bacilli in some part of the alimentary system, usually the gall-bladder. The results obtained, so far as published reports go, are not specially encouraging, but the treatment should be given a trial in all persistent cases.

#### ANTISTREPTOCOCCIC SERUM

Many of the complications of enteric fever are due to secondary invasion by pyogenic and other organisms. In this belief Clarke<sup>1</sup> has tried the effect of antistreptococcic serum in purulent periostitis occurring in enterica. He states that the use of this remedy was followed by a critical fall of temperature and a rapid cessation of the suppuration.

#### PARATYPHOID INFECTIONS

Cases also occur, identical clinically with those of enteric fever, but differing in their etiology, being due to one or other of the so-called "paratyphoid" bacilli (*B. enteritidis* of Gärtner; *B. paratyphosus* of Schottmüller, types  $\alpha$  and  $\beta$ ; or *B. paratyphosus* of Bryon and Keyser). In such cases the organism can usually be isolated by establishing cultivations in suitable media from blood (5–10 c.c.) collected in a sterile syringe from a vein in the arm. The most convenient guide to diagnosis in those who have not received prophylactic injections of antityphoid

<sup>1</sup> *Lancet*, 1899, i. 230.

vaccine is, however, the agglutination reaction, for *B. typhosus* is rarely clumped by the serum of these patients in any higher dilution than 1:20 (the clumping then being due to the presence of group-agglutinins), whilst the organism responsible for the infection is clumped in dilutions of 1:100 and 1:200, and even higher.

In cases which clinically resemble enteric fever, but fail to give a positive reaction to Widal's test, the agglutinating effect of the serum should be tried on both the varieties of paratyphoid bacilli ( $\alpha$  and  $\beta$ ), if these are available, and also on *B. enteritidis* of Gärtner.

Franchetti<sup>1</sup> has prepared an antitoxic serum in rabbits by injecting broth-cultures of *B. paratyphosus*- $\beta$ . The serum agglutinates this organism, but has not been used as a remedy in human disease.

**Antiparatyphoid inoculation.**—Castellani,<sup>2</sup> in view of the frequent occurrence of paratyphoid infections in the tropics, advocated prophylactic inoculation with "mixed" vaccines (containing the various bacilli in the following proportions—typhoid 500 millions, paratyphoid  $\alpha$  250 millions, and paratyphoid  $\beta$  250 millions per cubic centimetre) in doses of 0·6 c.c., followed by 1·2 c.c. seven days later, and, if possible, by a third dose of 1·2 c.c. a fortnight from the first. No statistics are available in proof of the value of this method, but, judging from the results of antityphoid inoculation, antiparatyphoid vaccine should be equally efficacious. Broughton-Alcock<sup>3</sup> has used paratyphoid vaccines, both "killed" and "living sensitized," and we gather that he has a preference for the use of the latter.

#### CONCLUSIONS

1. The bactericidal serum on the market, called "antityphoid," is generally unsatisfactory in its effects in cases of

<sup>1</sup> *Zeitschr. f. Hyg.*, 1908, lx. 128.

<sup>2</sup> *Ceylon Med. Repts.*, 1904-5.

<sup>3</sup> *Brit. Med. Journ.*, 1914, ii. 743.

enteric fever—indeed, it is doubtful whether it can be said to have any influence at all on the course of the disease.

2. Good results are reported with Chantemesse's anti-toxic serum, but there is not yet sufficient material on which to form an authoritative judgment as to its value.

3. Treatment by vaccines is even yet in the experimental stage, and no dogmatic opinion can be expressed as to its value.

4. The same criticism applies to Jez's antityphoid extract.

5. Wright's antityphoid inoculation constitutes a valuable measure of protection. It is not a certain means of preventing an attack of enteric fever, but it reduces the liability to this occurrence; while, if the disease does occur in an "inoculated" person, it is generally milder than in one not so protected. It seems advisable for young persons who are going to countries where enteric fever is rife to undergo prophylactic inoculation. There is no danger in the procedure, although it may entail twenty-four hours' discomfort.

6. Antiparatyphoid inoculation promises to yield equally good results, although at present the subject must be regarded as *sub judice*.

7. The Widal test (agglutination) for enteric fever is a useful means of clinical diagnosis. It is not infallible, but the margin of error is small—perhaps 5 per cent. An agglutination in a dilution of not less than 1:50 should be obtained before the result is called positive.

## CHAPTER XIV

### DYSENTERY AND OTHER BACILLARY INFECTIONS

#### DYSENTERY

APART from affections caused by the *Entamoeba dysenteriae* (*E. histolytica*),<sup>1</sup> the name dysentery is applied to cases of ulceration of the colon caused by a group of nearly allied organisms which share the title of *Bacillus dysenteriae*. The exact relationship of the members of this group is as yet uncertain. Varieties are distinguished by the names of their discoverers, as the Shiga bacillus, Flexner's bacillus, etc.

Mlle. Boïto<sup>2</sup> concludes from a study of agglutination experiments carried out with the various organisms that they fall into two groups—(1) that including the bacilli of Shiga, of Krüse, of Flexner (at New Haven), and others, which are agglutinated in dilutions of 1 : 400 ; (2) that including the bacilli found by Flexner at Manilla, by Hiss and Russell, by Krüse (asylums), and by some others, which have less agglutinative tendency. The question of the identity or diversity of the several organisms must be left at present unsettled.

**Toxins.**—Martin<sup>3</sup> found that by growing Shiga's bacil-

<sup>1</sup> Salvarsan has been given in tropical dysentery, with good results, by Matsuura (Abstr. in *Zeit. f. Immunitätsforsch.*, 1910, p. 1020), who used doses of 0.35 grm., and by Wadhams and Hill (*Journ. Amer. Med. Assoc.*, 1913, lxi. 385).

<sup>2</sup> *Gaz. des Hôp.*, 1903, No. 97, p. 781, and No. 80, p. 809. A full bibliography of the subject is here given. Cf. Gay and Duval, *Univ. of Penn. Med. Bull.*, July-Aug., 1903, p. 177.

<sup>3</sup> *Thirty-first Ann. Rept. of L.G.B.*, 1901-2; *Supplement containing the Report of the Medical Officer*, 1903, pp. 398, 402. Cf. Kraus and Doerr, "Das Dysenterio-toxin," Jena, 1907.

lus in peptone-broth a soluble poison was produced, which caused lowering of the temperature of animals into which it was injected; the animals also suffered from diarrhœa and loss of weight. The most potent part of the poison of the bacillus is, however, intracellular, the effect of injection of the dead bodies of the organisms being similar in kind to that of the toxins, but much more pronounced. The toxin is apparently innocuous if given by the mouth, but if injected into a vein it causes the typical lesions of the disease.

#### DIAGNOSIS

**Agglutination.**—This bacillus is agglutinated by the serum of patients who have recovered from the disease, or of animals inoculated with cultures. The serum of convalescents was found by Shiga to clump the bacilli in dilutions of 1 : 20 or 1 : 30. This property may remain for a considerable time after recovery has taken place (e.g. eight months), but the reaction does not appear at the beginning of the illness, so that it is of no importance for clinical diagnosis. Use has been made of it for the recognition of the bacillus, and for proving the identity of the varieties as described by different authors. In mild cases the reaction may never occur. Kruse found that in cases of dysentery agglutination might occur in dilutions of 1 : 50, and even of 1 : 1,000, while normal individuals never showed the phenomenon in greater dilution than 1 : 20. Flexner found the reaction present in cases due to his bacillus, whereas it did not occur in cases of amœbic dysentery.

Nicolle and Cathoire<sup>1</sup> find that agglutination in dysenteric infection is feeble, and that the agglutinins appear to differ according to the variety of bacillus present. On the other hand, Dopter<sup>2</sup> finds that the bacteriolytic copula formed in response to injections of the bacilli themselves is the same from all the strains.

<sup>1</sup> *Compt. Rend. Soc. Biol.*, 1906, No. 30.

<sup>2</sup> *Ann. Inst. Pasteur*, 1905, xix, 753.

Lucas, FitzGerald, and Schorer<sup>1</sup> have used the agglutination reaction as a means of diagnosis in infantile dysentery.

### SERUM TREATMENT

Shiga inoculated a horse with his bacilli, and obtained from it a serum which acted beneficially in cases of dysentery. He considered that by its use the mortality of the disease was reduced by nearly 50 per cent. Krüse considers that the serum prepared from his bacilli is bactericidal, not antitoxic. By the use of this remedy he obtained a fall of mortality from 10 to 8 per cent. These figures do not appear very striking. Shiga,<sup>2</sup> and also Coyne and Auché,<sup>3</sup> have prepared polyvalent serums by injection of bacilli belonging to all the different varieties. These serums are presumably antibacterial. Vaillard and Dopter<sup>4</sup> have prepared a serum by injection of both toxins and bacilli, and record good results (96 cases, with 1 death): the dose used was 20–100 c.c., repeated as necessary. Rosenthal<sup>5</sup> treated 157 cases, with 7 deaths, a mortality of 4·5 per cent. as compared with 10–11 per cent. in other German hospitals; the doses used were large (20–120 c.c.).

Kraus<sup>6</sup> gives the following figures illustrative of the value of serum treatment. In Galicia the mortality among 1,420 cases in which it was employed was 9·65, while among 6,914 cases treated otherwise the deaths amounted to 1·91 per cent. Similarly, in Bukovina the mortality was 9·11 per cent. among serum cases and 19·2 among those who received no serum. The correspondence of the figures is remarkable. The doses used were 30 to 40 c.c. subcutaneously.

<sup>1</sup> *Journ. Amer. Med. Assoc.*, Feb. 5, 1910.

<sup>2</sup> *Abstr. in Centraltbl. f. Bakt.*, I. Ref., 1908, xli. 742.

<sup>3</sup> *Compt. Rend. Soc. Biol.*, 1906, lx., No. 26.

<sup>4</sup> *Ann. Inst. Pasteur*, 1906, xx. 321; 1907, xxi. 241.

<sup>5</sup> *Deut. med. Woch.*, 1904, xxx., No. 19.

<sup>6</sup> *Ibid.*, 1912, No. 10.



Ruffer and Wildmore<sup>1</sup> prepared a serum by injecting horses first with bacterial cultures treated with pepsin and hydrochloric acid, afterwards with living organisms.

Subsequently (working in Egypt) they prepared different forms of polyvalent serum by inoculating horses subcutaneously and intramuscularly (1) with a mixture of different strains (Shiga, Krüse, Flexner, El Tor), and (2) separately with different strains of the El Tor and of the Shiga varieties. These various serums were then employed in the treatment of bacillary dysentery in the following manner: They examined the patients' serum for agglutinating properties towards these different varieties of dysentery bacilli, and, if one or other agglutinin predominated, used the corresponding serum; while for a preliminary dose, or in case of doubt, they used the former kind of serum—a "shot-gun" preparation against all known organisms responsible for the disease. The doses were 40–60 c.c. in mild cases, 80 c.c. in severe cases, and 100–120 c.c. in desperate instances. The results were encouraging, improvement in the patient's general condition usually ensuing in four to twelve hours, though the stools often remained apparently uninfluenced for some days. The serum was of no use in amœbic cases—an argument against the view still maintained by German writers that the amœbæ are saprophytes in an intestine already injured by the bacilli of dysentery.

Lesage<sup>2</sup> prepared a serum by inoculation with his cocco-bacillus, which reduced the mortality in the cases he observed by 50 per cent. Moreul and Rioux<sup>3</sup> also produced a serum by means of the variety of *B. coli* which they considered to be the causal agent in the cases they examined; they found that it was both preventive and curative.

**Prophylaxis.**—Castellani has advocated the prophylactic use of dysentery vaccine; and recently Broughton

<sup>1</sup> *Brit. Med. Journ.*, 1910, ii. 1519.

<sup>2</sup> *Bull. de la Soc. de Biologie*, 1902, p. 705.

<sup>3</sup> Quoted by Mlle. Boïto, *op. cit.*

Alcock<sup>1</sup> has employed for the same purpose a sero-vaccine in which an inactivated normal serum (human or equine) is used precisely as if it were an immune serum, to treat the bacilli, and claims for the process abolition of toxic effects of the vaccine and the production of a higher grade of immunity.

#### VACCINE TREATMENT

In cases of chronic dysentery, rebellious to other treatment, Forster<sup>2</sup> found benefit to accrue from the use of a vaccine of dead organisms. He used cultures of Shiga's bacillus heated to 60°–63° C. and suspended in salt-solution. The value of this treatment is confirmed by Stephens<sup>3</sup> and by Newman.<sup>4</sup>

Dopter<sup>5</sup> has shown that vaccine prepared from cultures of Shiga's bacillus and treated with specific antiserum gives an early and prolonged immunity in experimental animals.

#### INFANTILE ENTERITIS, OR SUMMER DIARRHŒA

The *Bacillus dysenterice* has been found in cases of the summer diarrhœa of infants, and is considered by some writers to be the cause of the disease.<sup>6</sup> Morgan<sup>7</sup> has isolated a bacillus, "No. 1," from some 63 per cent. of the cases which he investigated, and regards it as the causal agent. It is probable that more than one bacterial agent can give rise to the morbid conditions classed under this heading. Krüse obtained good results from serum treatment in cases of dysentery in children, reducing the

<sup>1</sup> *Brit. Med. Journ.*, 1914, ii. 306.

<sup>2</sup> *Ind. Med. Gaz.*, 1907, p. 201.

<sup>3</sup> *Ibid.*, p. 375.

<sup>4</sup> *Lancet*, 1908, i. 1410.

<sup>5</sup> *Ann. Inst. Pasteur*, 1909, xxiii. 676.

<sup>6</sup> See Martha Wollestein, "The Dysentery-Bacillus in a Series of Cases of Infantile Diarrhœa," *Journ. of Med. Research*, Aug., 1903, p. 11.

<sup>7</sup> *Proc. Roy. Soc. Med.*, March, 1909.

mortality from 15 to 5 per cent. Gay<sup>1</sup> considered that the prospect of serum treatment in summer diarrhoea was very hopeful; but the reports of the Rockefeller Institute on the use of antidyenteric serum in this affection are not encouraging.<sup>2</sup>

### COLITIS

Membranous, mucous, and ulcerative colitis are terms employed to denote a condition or group of conditions which some observers consider to be identical with bacillary dysentery, especially the cases which occur in asylums for the insane. Sporadic cases of long standing have been found by Halc White and Eyre<sup>3</sup> to be amenable to treatment with autogenous vaccines prepared from *B. coli communis* variants, especially a coliform bacillus which does not ferment lactose present in the intestine and faeces of patients, and which Eyre believes to be causally associated with the condition. Similarly, *Streptococcus longus* or pneumococci may sometimes be found in the dejecta of the hæmorrhagic types of colitis, and in these cases treatment with autogenous vaccines usually gives good results. In view of the intractable nature of the conditions, trial of vaccine treatment is certainly to be recommended before adopting the *ultimum refugium*—now so often the first thought of the medical attendant—a surgical operation.

### AFFECTIONS DUE TO BACILLUS COLI COMMUNIS

The *Bacillus coli communis* is a normal inhabitant of the human intestine. Under certain circumstances, such as constriction of the gut, injury to the peritoneum, perforation of the intestine, etc., it is capable of giving rise to serious symptoms; it may also be found in suppurative conditions, such as pyelitis and cystitis, otitis media, etc., attended sometimes by considerable elevation of temperature; in all of these it acts as a pyogenic organism.

<sup>1</sup> *Univ. of Penn. Med. Bull.*, Nov., 1902.

<sup>2</sup> See *Brit. Med. Journ.*, 1904, i. 1653.

<sup>3</sup> *Lancet*, 1909, i. 1586.

*B. coli* alone may also give rise to a true septicæmia, and is frequently associated with *Streptococcus longus* in the causation of puerperal septicæmia, thus explaining the occasional failure of antistreptococcic serum to check the course of that infection. This bacillus appears sometimes to have some association with Graves's disease, since we have found in several cases that the inoculation of small doses of autogenous *B. coli* vaccine has been followed by decrease in the objective signs of the disease, whilst a large dose has been promptly followed by increase in the size of the thyroid accompanied by distressing palpitation.

**Toxins.**—Carega<sup>1</sup> has isolated two toxic substances from broth-cultures of these bacilli—a nuclein and a nucleo-albumin: to the latter, no antibody is apparently formed in injected animals.

Attempts to prepare an antitoxic or bactericidal serum against this organism have not been successful in the hands of Albarran and Mozer;<sup>2</sup> but antisera to the *Bacillus coli* are still on the market.

**Vaccine treatment** has proved of great value. Thus, Wright<sup>3</sup> caused the closure of a sinus left after operation by means of a vaccine of the organism; and this procedure was also used by Hawkins and Corner<sup>4</sup> in a case of infection following laparotomy for appendicitis. Gray<sup>5</sup> has used coli vaccine with success in cases of pyelitis; and Hicks<sup>6</sup> reports favourably on its use in the treatment of the pyelitis of pregnancy. Good results are reported in two cases of cholecystitis, the dose of vaccine being 100 to 200 millions of dead bacilli.<sup>7</sup> We have found the

<sup>1</sup> *Centralbl. f. Bakt.*, 1903, xxxiv., No. 4.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, Jan. 17, 1899.

<sup>3</sup> *Trans. Path. Soc. London*, 1906.

<sup>4</sup> *Brit. Med. Journ.*, 1908, ii. 782.

<sup>5</sup> *Lancet*, 1906, i. 1102.

<sup>6</sup> *Brit. Med. Journ.*, 1909, i. 203.

<sup>7</sup> Wright and Reid, *ibid.*, 1906, i. 143.

treatment of great value in cases similar to the above; also in cystitis, subdiaphragmatic abscess, etc. The vaccine must be prepared from the patient's own organism, for in coli-infections stock vaccines are useless. The initial dose is usually 2·5 to 5 millions, repeated in from two to three days. Subsequent doses are generally given at intervals of about a week in increasing quantities up to a maximum of 50 millions. It will often be found that after a period of improvement the patient ceases to respond to the injections of vaccine, and on investigation it will sometimes be found that the colon bacillus still infecting the individual has varied slightly in its biological character; if that is so a fresh vaccine prepared from this new strain will often complete the cure. At other times recourse to a sensitized vaccine will have the same effect.

#### AFFECTIONS DUE TO BACILLUS PYOCYANEUS

The blue coloration of pus, particularly that so often noted as following extensive burns, is due to *Bacillus pyocyaneus*, called also the "bacillus of blue pus."

**Toxins.**—MacIntyre<sup>1</sup> found that the bodies of the bacilli contained an intracellular toxin and a hæmolysin.

**Antitoxic serum.**—Wassermann obtained both an antitoxic and a bactericidal serum by injection respectively of the toxins and of the bacillus itself into animals. These serums had both a protective and a curative action on animals.

We have found *vaccine treatment*, in doses of 50 and 100 millions, of value in cases of osteomyelitis, shrapnel wounds, and suppurating sinus following laparotomy for ovarian tumour.

An extract of the bacilli was prepared by Emmerich and Low<sup>2</sup> by autolysis, and is called "pyocyanase." It seems to act as a bactericidal agent not only on *B. pyocyaneus* but also upon other pathogenic bacteria. It has been used by

<sup>1</sup> *Journ. Amer. Med. Assoc.*, April, 1904, p. 1074.

<sup>2</sup> *Zeitschr. f. Hyg.*, 1899, xxxi. 1; 1901, xxxvi. 9.

Hofbauer<sup>1</sup> in cases of vaginal gonorrhœa, with only transitory benefit; and by Escherich<sup>2</sup> as a local disinfectant in nasal catarrh due to *Micrococcus catarrhalis*, and in cerebro-spinal meningitis. In the former affection it was very successful, but in the latter its value was not definitely proved. It has also been used as a local application to the false membrane in diphtheria and in ulcer molle. In this last the application is a somewhat painful procedure.

#### YELLOW FEVER

**Etiology.**—The causation of yellow fever is at present unsettled. Sanarelli<sup>3</sup> isolated a bacillus to which he gave the name of *Bacillus icteroides*, and which he believed to be the pathogenic agent. The claims of this organism, however, have not been supported by other observers. Parker, Beyer and Pothier<sup>4</sup> have found a protozoan organism which they named *Myxococcidium stegomyiae*, and which is possibly the cause of the disease. This coccidium is found by them in the bodies of gnats which have sucked the blood of patients suffering from yellow fever. It is now practically proved that the disease is transmitted by the bites of the variety of gnat called *Stegomyia fasciata*, and Findlay<sup>5</sup> suggests that this insect is the principal host of the parasite of yellow fever, which only passes a subordinate stage of its existence in human beings. Its life-cycle would thus be just the opposite of that of the organism of malaria, which has man for its definitive host. It is, however, more than probable that the true cause of the disease still remains to be discovered.

**Toxins of *B. icteroides*.**—Baker<sup>6</sup> injected toxins derived from *Bacillus icteroides* into patients, and found that

<sup>1</sup> *Centralbl. f. Gynak.*, 1908, No. 6.

<sup>2</sup> *Wien. klin. Woch.*, 1906, xix. 751.

<sup>3</sup> *Ann. de l'Inst. Pasteur*, 1897, xi. 433.

<sup>4</sup> *United States Public Health and Marine Hospital Service (Yellow Fever Bulletin, No. 13)*, 1903.

<sup>5</sup> *Rivista de Medicina Tropical*, 1903, No. 4, p. 49.

<sup>6</sup> See *Journ. Amer. Med. Assoc.*, April 14, 1900.

they produced the typical phenomena of a rising pulse and falling temperature (Faget's pulse and temperature), which are peculiar to this disease.

**Serum treatment.**—Sanarelli<sup>1</sup> prepared an "anti-amarillic" serum<sup>2</sup> by inoculating a horse with his bacilli, and used it in cases of the disease, apparently with good effect. He reports that the injection is followed by a febrile reaction, which in turn is succeeded by remission of the symptoms. Among 8 cases in which he used small doses of the serum there were 2 deaths and 6 recoveries; while of 14 severe cases in which larger quantities were employed, 10 recovered.

*Prophylactic* injections of the serum were used in the case of an outbreak which had occurred in a jail, and after the injections were carried out no more cases of the disease were met with.

Agramonte<sup>3</sup> has tried the *serum of convalescents* in yellow fever, and thinks that good effects are produced. Other observers have not been equally successful.

**Vaccination** with the *Bacillus icteroides* in accordance with Haffkine's method is capable of protecting animals against infection with this organism.

## WHOOPIING-COUGH

Various organisms have been claimed by different observers as the causal agent of whooping-cough, but, despite the effect of certain so-called specific serums referred to below, the bacillus described by Jochmann, Bordet, and others possesses the strongest claims to recognition. The *Bacillus pertussis* is a minute slender rod belonging to the influenza group of hæmophilic bacteria; that is to say, it does not retain the stain by Gram's

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1898, xii. 348.

<sup>2</sup> Amarillic, from the Spanish name of the disease, "fiebre amarilla."

<sup>3</sup> Quoted by Fitzpatrick, *Journ. of the American Med. Assoc.*, April 14, 1900, p. 905.



method, will only develop in artificial media containing blood or serum, and only at the temperature of the body.

**Serum treatment.**—Elena Manicatide<sup>1</sup> obtained pure cultures of a peculiar bacillus from the sputum of patients, and with them inoculated a sheep and a horse. The serum obtained from these animals appeared to exert a favourable influence on the disease, the number of attacks diminishing under the treatment, and convalescence being more rapid.

Leuriaux<sup>2</sup> found in cases of whooping-cough a short, thick bacillus, almost as broad as it was long. It was motile and aerobic, growing well on gelatin and other laboratory media; and it retained the colour when treated by Gram's method. He inoculated rabbits with this organism, which produced death if it was given intravenously, while if it was given subcutaneously a local abscess was formed. Convulsive movements of the diaphragm were seen in some instances, which may be analogous to the convulsive seizures of pertussis. Leuriaux inoculated a horse with the organisms, and treated cases of whooping-cough with its serum. He considers that the effects produced were good, cure being brought about in six to eight days. He employed doses of 5 c.c., and advises the early administration of the remedy.

It is noteworthy that these two observers agree in finding a specific bacillus and in obtaining good results by serum treatment. It is impossible to know whether the organism isolated was the same in both cases. Much further experimental evidence is necessary, both as to the specificity of the bacillus and as to the efficacy of the serum, before we can conclude that a true remedy has been discovered for this malady. The course of the disease is so irregular, and the difficulty of judging of the value of any treatment is so great, that a considerable degree of scepticism is justifiable in the case of any new "cure" which is brought forward.

<sup>1</sup> *Spitalul*, 1902, No. 6 (abstr. *Centralbl. f. inn. Med.*, 1903, p. 199).

<sup>2</sup> *La Semaine Méd.*, 1902, p. 233.

Silvestri<sup>1</sup> used injections of the *serum of convalescents* (15–20 c.c.) in 7 cases of whooping-cough, all of which were apparently relieved.

**Non-specific treatment.**—Indica<sup>2</sup> and other observers speak favourably of the use of diphtherial antitoxin in whooping-cough. Indica treated 8 cases with this serum, which he considers to act by stimulating leucocytosis and increasing the resistance of the patient.

Porchi<sup>3</sup> thinks that vaccination (vaccinia) is both prophylactic and curative.

**Vaccine treatment.**—Some writers record their experiences with vaccines of the Bordet-Gengou bacilli. Thus, Bamberger<sup>4</sup> gave doses of 20 millions of these organisms in 6 cases, and thought that the severity of the symptoms was reduced but the duration of the illness was not lessened. Scott<sup>5</sup> gave doses of 40 millions even to infants, and arrived at very similar conclusions as to the effects of the treatment; and Graham<sup>6</sup> with these doses noted apparent improvement in 17 of 24 cases. Ladd,<sup>7</sup> however, found the procedure useless.

Nicolle and Conor<sup>8</sup> used a vaccine of living organisms (Bordet-Gengou), administering 1 to 5 minims of a suspension containing 400 millions of the organisms in 1 c.c. saline solution. They do not seem to have been favourably impressed with the results attained.

## BOTULISM

The word “botulism,” originally applied to any form of “sausage poisoning,” is now confined to affections produced

<sup>1</sup> *Gaz. degli Ospedali*, 1903, No. 114.

<sup>2</sup> *Ibid.*, 1900, xxi. 968.

<sup>3</sup> *Ibid.*, 1903, No. 114.

<sup>4</sup> *Amer. Journ. Dis. of Children*, 1913, v. 33.

<sup>5</sup> *N.Y. Med. Journ.*, Jan. 25, 1913.

<sup>6</sup> *Med. Record*, 1911, lxxx. 402.

<sup>7</sup> *Arch. of Pediatr.*, 1912.

<sup>8</sup> *Compt. Rend. Acad. Sci.*, 1913, clvi. 1849.

by eating meat contaminated with a particular micro-organism, the *Bacillus botulinus*, discovered by van Ermengem.

By growing the bacilli in broth and filtering the cultures, Kempner<sup>1</sup> obtained a toxin which, when injected into goats, produced an antitoxic serum. This was found capable of protecting rabbits against many times the lethal dose of the toxin, and also of exerting a curative effect if given some hours after the poison. No records of the use of this serum in human beings are available.

The toxin gives rise in the cells of the spinal cord to changes of a degenerative nature, similar to those found in fatal cases of the disease.

<sup>1</sup> *Zeitschr. f. Hyg.*, 1897, Bd. xxvi.

## CHAPTER XV

### TUBERCULOSIS

#### ETIOLOGY

**Causal bacillus and toxins.**—The *Bacillus tuberculosis* was discovered by Koch in 1882. By growing the bacteria in glycerin-bouillon some of the toxins may be obtained, but the bodies of the bacilli are themselves toxic, acting as distinct irritants even when they are dead. The toxins of the tubercle bacillus are not well understood. A peculiar acid formed by it has been credited with the power of producing the characteristic degeneration of the tissues called “caseation.” Another substance gives rise to fever; and certain volatile products are said to have a convulsant effect. The bacilli may cause death in two ways—either by rapid multiplication and formation of toxins, as in acute miliary tuberculosis; or by producing gradual destruction of some organ necessary to life, such as the kidney or the lung. In these latter cases there is often a super-added infection by pyogenic organisms, staphylococci, etc., to the toxic effects of which the profuse sweats so characteristic of advanced tuberculosis are principally due, and very possibly also much of the ulcerative destruction of tissue met with in cavities in the lungs as well as in superficial lesions.

The question of the identity or difference of human and bovine bacilli is still undecided. Professor Koch held that the organisms were distinct, and that bovine tubercle could not be conveyed to man, nor human tubercle to cattle. The general opinion at the present time appears to be that there is no such absolute distinction between them, although

the difficulty of infecting bovine animals with human bacilli is admitted to be very great. Birds suffer from a special form of tubercle, which appears to be caused by a distinct, though nearly allied, organism, and cold-blooded animals (snake, tortoise) also suffer from an infection produced by an acid-fast bacillus. Use has been made of the different varieties of the *B. tuberculosis* for the purpose of immunizing animals, inoculation with bacteria of the kind special to another animal not producing tuberculosis, but rendering the animal inoculated immune against that form of the disease from which it is liable to suffer.

#### TUBERCULIN

**Artificial preparation of the toxins of the tubercle bacillus.**—Koch found that by growing the bacilli from six weeks to two months in flasks containing slightly alkaline veal-broth, to which a percentage of peptone and of glycerin had been added, and freely supplying the cultures with oxygen throughout, it was possible to obtain a fluid containing some, at any rate, of the toxins produced by the organisms. By passing this through a porcelain filter the bodies of the bacteria were removed, and a solution of the poisons remained. This was concentrated by evaporation to one-tenth of its bulk, and to the fluid thus obtained the name of *tuberculin* (T. or O.T., Old or Original Tuberculin; sometimes A.T., Alt Tuberculin) was given. It was hoped that this preparation would exert a curative effect on tubercular disease (phthisis), and the discovery was announced to the world as a “cure for consumption.” Great hopes were thus raised, only to be dashed to the ground when further experience was gained as to the limitations of the method and the inconstancy of the good results produced. The reaction which ensued as the result of this disappointment undoubtedly went too far in the opposite direction, and the limited, but really valuable, properties of tuberculin as an aid to treatment were overlooked or denied.

**Composition of tuberculin.**—Tuberculin, as thus prepared, is a somewhat thick fluid, of dark-yellow colour. It is practically a solution in glycerin of the substances, toxic and other, formed by the bacillus during its growth in a fluid medium, since the glycerin added to the original culture-medium does not evaporate on heating, while the water is driven off. A careful analysis of tuberculin, as originally prepared by Koch, was made by W. Hunter,<sup>1</sup> as the result of which he came to the conclusion that it was a very complex substance. He separated from it (besides glycerin, etc.) alkaloids, albumoses, and extractives. He considered that the material which produces the febrile reaction was of the last class, and, as these matters are separable by dialysis, he hoped to produce a tuberculin free from the objectionable features of Koch's preparation. The remedial substance is, in his view, probably an albumose, as is also that producing the inflammatory reaction around the foci of tuberculosis. There are thus at least three active principles present in tuberculin.

Koch, as the result of independent analysis, agreed in considering that the most important material was of the nature of an albumose, but he was doubtful as to its exact chemical nature, owing to its power of resisting high temperatures—a peculiarity which Hunter also had noted.

**Koch's later tuberculins.**—In addition to the above tuberculin, Koch<sup>2</sup> subsequently introduced other remedial preparations of tubercle bacilli. (1) Instead of the toxin produced by growth of the bacilli in a fluid medium, he made use of extracts of the organisms themselves. He took highly virulent cultures of tubercle bacilli, dried them *in vacuo*, and triturated them in a mortar. The resulting powder was treated with sterile distilled water, and submitted to centrifugalization. The supernatant clear, but opalescent, fluid was then removed from the débris, and 20 per cent.

<sup>1</sup> *Brit. Med. Journ.*, 1891, ii. 169.

<sup>2</sup> *Deut. med. Woch.*, 1897, p. 209.

of glycerin added as a preservative. To it Koch gave the name of Tuberkulin-O (T.O.), or "Oberer (Upper) Tuberkulin." This corresponds in composition and action with the original tuberculin, T. (2) The solid residue thus freed from soluble toxins was then dried, and the process of extraction by triturating with 20 per cent. glycerin-solution and then centrifugalizing was repeated several times, the fluid used each time being preserved, and the whole finally mixed together. This mixture constituted the New or Residual Tuberculin (*T. rückstand*, T.R.). Koch claimed that specially valuable properties reside in this last preparation, gradually increasing doses injected into animals producing immunity to tuberculosis, and also to the action of the other forms of tuberculin. (3) A third modification (T.A., *Tuberculin alkalinum*) is obtained by extracting dried tubercle bacilli with decinormal soda-solution, and filtering the fluid. Tuberculin-O and Tuberculin-A produce effects very similar to those of the Old Tuberculin (T.). (4) A fourth modification, known as "New-Tuberculin B.E." (bacillus emulsion), is merely a suspension of 5 mg. of powdered tubercle bacilli in 1 c.c. of 50 per cent. solution of glycerin, and is the equivalent of an ordinary bacterial vaccine.

All the preparations previously mentioned are manufactured from *B. tuberculosis* of the human type. Precisely similar preparations are made from *B. tuberculosis* of the bovine type, and these are indicated by prefixing the letter P. (Perlsucht) to the letter or combination of letters which stand for those prepared from human tubercle bacilli.

**Action of tuberculin.**—It is found that if a minute quantity of the *original preparation* (old tuberculin) is injected hypodermically into a patient or animal suffering from tuberculosis, very definite symptoms are produced. There is a rise of temperature of varying intensity, from one to three or more degrees Fahrenheit, accompanied by a feeling of illness and sometimes by nausea or even



vomiting. At the seat of any localized focus of tuberculosis which is open to observation there occurs a more or less vigorous reaction, with heat and redness; and often, if this is severe, there is a casting-off of necrosed pieces of tissue.

The mode of production of the fever, in the case alike of the old and of the new tuberculin, is not well understood. It cannot be due to the existence in the tuberculin of a direct thermogenic substance, as in that case normal individuals would be affected in the same way as the tuberculous. It has been suggested that in the case of the old tuberculin the fever is the result of the local inflammation excited around the lesions, but this is doubtful in view of the similar action of the new preparation, which is not followed by any such local effects. We are driven to suppose that there is an interaction between two substances, one contained in the tuberculin and the other present in the body of the tuberculous individual, the result of which is the formation of some pyogenic substance as yet unknown.<sup>1</sup> The explanation put forward by Ehrlich is as follows: The normal cells of the body are not affected by this substance, nor are those which form the actual tubercular tissue. Probably the latter are habituated to the poison, as they are in close relation with the bacilli which are constantly giving rise to its formation. There is, however, a zone of cells at a certain distance from the centre of infection which have been only so far affected by the poisons of the bacillus as to be rendered unusually susceptible to their influence. When an injection of tuberculin is administered, an additional quantity of poison is brought into contact with these cells, and they are thus stimulated to react. The reaction takes the form of inflammation—the process by which dead or dying tissues are cast off from the body, as is seen in the separation of a sequestrum or a

<sup>1</sup> Krehl and Matthes found that albumoses derived from many different sources produced effects precisely similar to those of tuberculin. (*Arch. f. exper. Path. u. Pharmac.*, 1895, xxxvi. 437.)

slough. Hence the necrotic tubercular tissues tend to be cast off by the action of the tuberculin, and a more or less healthy granulating surface is left in favourable cases.

The anatomical effects produced in a tuberculous subject by an injection of tuberculin may be best seen in an infected guineapig which has been killed by the injection of a large dose of this substance. A zone of hyperæmia may be seen surrounding each of the grey nodules characteristic of the disease, which occur throughout all the internal organs. Healthy guineapigs can tolerate even large doses of tuberculin without manifesting any symptoms; tuberculous animals are killed by a moderate dose. Human beings are apparently more susceptible to tuberculin than are guineapigs.

These facts tend to show that at all events a possible source of danger resides in this substance when it is used on tuberculous patients. They also seem to prove that, as is the case with the fever-producing substance noted above, tuberculin does not contain a substance directly poisonous in itself, but rather contains some material which interacts with another substance present in infected individuals, the two together forming a poison. The reaction would seem to be analogous to the phenomena of anaphylaxis described on p. 20, though certain small differences have been said to exist between them.

After the reaction has subsided, it is seen in many cases that an improvement in the local disease has set in, with a tendency to healing in what may have previously been an indolent sore. The tubercular disease of the skin known as lupus vulgaris is the form of tubercle in which this result is best seen, but the same phenomena may take place in any focus of the disease.

There is no doubt of the fact that some degree of immunity to tubercular infection can be produced in animals by injections of tuberculin. Of its action on human beings it is possible to obtain some indication by the increased power

of agglutinating the *Bacillus tuberculosis*, seen in the blood-serum of such patients (*see* p. 289), and by its effects on the opsonic index. It is not, indeed, certain that the immunity of the individual is directly proportionate to either of these properties, but there is evidence to suggest that they constitute some measure of the resistance. It seems, however, that as a result of injections of tuberculin an individual may acquire a tolerance of this drug without an accompanying immunity to tuberculosis.

The action of the *new tuberculin* (T.R.) is quite different, as far as can be observed. The injection of a small quantity of this—the actual substance of the bacteria—causes, indeed, a general reaction of a febrile nature in tuberculous patients, but this is unconnected, as far as can be seen, with changes at the site of existing lesions. The curative effect of the injections is exerted by stimulating the tissues of the body generally to form antibodies to the tubercle bacilli. In other words, the new tuberculin is supposed to give rise to a condition of general immunity. It certainly seems to have the power of raising the agglutinating power of the serum, and also its opsonic properties, and there is reason to believe that these increase *pari passu* with its antibacterial properties.

Marmorek maintains that an interaction takes place between the tubercle bacilli themselves and the tuberculin; and states that a febrile reaction may occur if tuberculin is injected almost immediately after inoculation of bacilli. This statement, however, is not confirmed by other observers.

Patients in the earlier stages of tuberculosis appear to react to tuberculin more strongly than those in whom the disease is more advanced; indeed, those in the third stage of phthisis may fail to give any reaction. This may be due to the fact that their tissues have become habituated to the toxins. It does not detract from the practical diagnostic value of the drug, since it is in the early stages especially that the disease is difficult to recognize.

**Emulsion of bacilli.**--Koch,<sup>1</sup> as we have seen (p. 273), modified his procedure in the matter of inoculation in tuberculosis, and preferred to use, instead of the toxins of the bacilli or an extract of their body-substance, the actual bacilli themselves. Powdered tubercle-bacilli are suspended in 50 per cent. solution of glycerin, and the fluid is allowed to stand till all particles of any appreciable size have sunk to the bottom. The supernatant emulsion is poured off, and is ready for use. The quantities are adjusted so that 1 c.c. of the fluid contains 5 mg. of powdered bacilli. For use it is diluted with normal solution of sodium chloride. Koch started with subcutaneous injections calculated to contain 0.0025 mg. of the solid material. He repeated the dose every two days or so, raising the quantity administered each time to twice, or even five times, the amount of the previous dose. No reaction occurs as a rule with the first small doses. It may occur as the dose is raised, and when it is observed the intervals between the injections must be prolonged (eight days or so).

The agglutinative power of the serum in patients so treated rises rapidly in the great majority of instances. In a minority it remains stationary, or may even sink. In such cases Koch gave intravenous injections of a fluid corresponding with his earlier T.O. (see p. 273), but only very small doses can be administered in this way. Koch considered, however, that the intravenous method has great advantages; and after introducing it he in many cases started with the subcutaneous mode of injection, and had recourse to the intravenous method afterwards as soon as a reaction occurred with the former.

Koch found that with this treatment the patients gained appetite and weight, night-sweats ceased, moist sounds disappeared from the lungs, and the amount of sputum was reduced. The presence of fever is not a contraindication to this treatment; indeed, pyrexia is reduced by it.

<sup>1</sup> *Deut. med. Woch.*, Nov. 25, 1901.

## MODIFICATIONS OF TUBERCULIN

Roughly, the different forms of tuberculin enumerated below may be divided into—

Group I. Cultures and bacterio-proteins.

„ II. Unheated tuberculins.

„ III. Modifications of tuberculin produced by chemical agents, including autolysates.

It is generally believed that all tuberculins contain the same active substance, the different preparations varying in concentration and in the nature of their other constituents.

## GROUP I

## BOVINE TUBERCULIN

Spengler and Raw<sup>1</sup> have advised the use of a tuberculin derived from cultures of bovine bacilli (“Perlsucht Tuberculin”: P.T.), either in place of the ordinary preparation or in conjunction with it (*see* p. 273).

## VON RUCK'S TUBERCULIN

This is a watery solution or extract<sup>2</sup> of tubercle bacilli, comparable with Koch's Oberer Tuberkulin (T.O.). It has been used in the United States with good results (p. 327).

## VON RUCK'S PROPHYLACTIC

Von Ruck<sup>3</sup> has devised a method of active immunization against tuberculosis by means of a preparation made as follows: An old and non-virulent culture of tubercle bacilli is washed and macerated with water containing 0.4 per cent. phenol; it is then filtered through porcelain, the filtrate, containing some proteose and peptone,

<sup>1</sup> *Deut. med. Woch.*, 1908, No. 38.

<sup>2</sup> *Therapeutic Gaz.*, 1896, p. 308.

<sup>3</sup> *Med. Record*, 1912, lxxxii. 369.

constituting protein No. 1. The bacillary mass is further washed and extracted with ether, dried again, powdered, and extracted with water, the filtrate being protein No. 2. A third process of powdering, extracting, and filtering gives protein No. 3; and the remnant is treated with 0.4 per cent. solution of sodium hydrate, and filtered, producing protein No. 4. The vaccine consists of 0.25 parts of No. 1, 2.75 parts of No. 2, 1 part of No. 3, and 6 parts of No. 4, and contains 10 mg. of solid substance in 1 c.c. The dose for children is 0.2 c.c. to 0.6 c.c., for infants 0.05 c.c.

#### TUBERCULOPLASMIN

Hahn,<sup>1</sup> by squeezing the bacilli in a hydraulic press, according to Buchner's method, prepared a fluid to which he gave the name of "tuberculoplasmin." We have not been able to find any record of its employment in therapeutics.

#### BACILLOSINE

Levet<sup>2</sup> grows the bacilli for three months and then distils the cultures. It is not clear from his account whether he then uses the distillate or the residue for immunizing purposes—presumably the former. The preparation is made in several strengths—No. 0, 1 per cent.; No. 1, 2 per cent.; and No. 2, 3 per cent. It may be used as a prophylactic.

#### TUBERAL

A remedy sold under this name is said to be an albuminous substance (tuberculo-albumin) derived from the bodies of tubercle bacilli. It is a greyish-white substance, and for use is dissolved in a 0.3 per cent. solution of phenol. It is administered by the mouth in doses of 10 to 40 drops. We have not been able to find the article in which it was originally described by its inventor, Thamm.

<sup>1</sup> Quoted by Shaw, *Lancet*, 1908, i. 926.

<sup>2</sup> *Arch. Gén. de Méd.*, 1906, cxvii. 965.

## TULASE (TUBERCULASE)

Behring announced the preparation of an extract of tubercle bacilli (tulase) from which toxic matters have been removed and which he believes will be useful for treatment and prophylaxis. The exact manner in which the substance is prepared has not been published, and so far the remedy is not generally procurable. Collin,<sup>1</sup> who used some of it in certain cases of ophthalmic tuberculosis, speaks of it as a wax-like residue which separates from the bacilli when they are rubbed up with chloral hydrate and left to stand. The doses given were 0.01 mg. to 8 mg. Tulase does not appear to have been employed in the treatment of any large series of cases. An immune serum or *antitulase*, prepared by injecting animals with tulase, may also be used to induce a passive immunity. Nothing has been heard of this preparation for the last few years.

## BRUSCHETTINI'S SERO-VACCINE

Bruschettini<sup>2</sup> prepared an antitubercular serum by injecting horses with successive doses of (a) bacilli killed by a temperature of 60° C. ; (b) of endotoxin derived by extraction of bacilli with 0.5 per cent. phenol solution ; (c) of bacilli which had been placed in collodion sacs within the peritoneal cavities of immunized animals ; (d) of an extract of the lungs of rabbits which had received injections of tubercle bacilli and of endotoxin, and of (e) a leucocytophil substance ; and finally (f) of living virulent bacilli. The serum from these horses was mixed with bacilli which had been kept in contact with leucocytes—presumably in the pleural cavities of rabbits or guineapigs. This sero-vaccine (sensitized vaccine) was tried at Brompton Hospital by Hector Mackenzie and A. C. Inman, but, though Bruschettini

<sup>1</sup> *Münch. med. Woch.*, 1907, No. 36.

<sup>2</sup> Internat. Congr. of Tub., Brussels, 1910 ; XVIIth Internat. Congr. of Med., London, 1913 ; XIth Internat. Anti-Tub. Conf., Berlin, 1913.



speaks warmly of the results achieved, Inman<sup>1</sup> was unable to discover any valuable effects. A modified form of the vaccine is also prepared for separate use.

#### DIXON'S TUBERCULIN<sup>2</sup>

Mixed human and bovine cultures are dried, washed several times in ether to remove the fatty coat, ground in a mortar, and suspended in saline solution (1 part in 5 of fluid). The suspension is well shaken for several hours and then allowed to stand for several days, after which it is filtered free of organisms. The stock solution contains 0.5 gm. of bacillary substance in 1 c.c. The doses advised are 0.001, 0.01, 0.02 gm., and so forth, given every five or seven days. Francine and Hartz<sup>3</sup> speak well of this preparation in chronic cases of pulmonary disease.

#### FRIEDMANN'S TUBERCULIN

Friedmann<sup>4</sup> made a communication to the Berlin Medical Society in November, 1912, in which he stated that he had had good results in the treatment of tuberculosis by injection of a vaccine consisting of living bacilli derived from tubercular lesions in cold-blooded animals. His paper was said to be founded on the experience gained in application of the remedy in 1,012 cases. Favourable opinions were expressed by some who had tried the preparation—Karfunkel, Schleich, Müller, Thalheim, and others. It is given hypodermically or intramuscularly, and causes the appearance of local induration and sometimes of abscess-formation. Later experiences with this tuberculin have not been encouraging (Mannheimer,<sup>5</sup> Barnes<sup>6</sup>), and a journey to the United

<sup>1</sup> Personal communication.

<sup>2</sup> Dixon, *Pennsylv. Health Bull.*, Oct., 1911.

<sup>3</sup> *Journ. Amer. Med. Assoc.*, March 8, 1913.

<sup>4</sup> *Berl. klin. Woch.*, 1912, xlix. 2214, 2241.

<sup>5</sup> *Ibid.*, 1913, p. 1301.

<sup>6</sup> *Providence Med. Journ.*, Nov., 1913.

States undertaken by Friedmann to demonstrate his preparation was far from leading to acceptance of his claims in that country. It is said that in some cases which died there was found a miliary tuberculosis of the muscles near the site of injection—a condition from which alarming inferences have been drawn as to the possible danger inherent in the use of this tuberculin. It must, however, be borne in mind that dead bacilli may give rise to tubercles where they settle, and living bacilli of foreign type might theoretically also do so and yet not possess the power of causing a spreading generalized tubercular infection. The preparation is stated to be quite harmless to guineapigs.

A very similar remedy was prepared by Piorkowsky, who lectured on it in this country and presented some of it for trial at the Brompton Hospital. It consisted of a filtrate corresponding with Old Tuberculin and a suspension of bacilli corresponding with B.E., both obtained from cultures of bacilli derived from a turtle. Trial in a few cases at the above hospital did not produce a favourable impression as to its value. The routine advised was the administration subcutaneously or intramuscularly of two doses of suspension of bacilli (0·5 and 1 c.c. respectively) at a week's interval, followed by injections of the filtrate 0·1, 0·2, 0·5 c.c., and so forth. No good effects were observed. In one case an abscess formed some three months after the injection. Another patient, who left the hospital improved to some extent, was readmitted within a few weeks, and died with an acute broncho-pneumonic tuberculosis.

#### ROSENBACH'S TUBERCULIN

This preparation is made by growing *Trichophyton holosericum* on cultures of tubercle bacilli (six to eight weeks old) in glycerin-broth at 20°–22° C. for a period of ten to twelve days; separating the growth, which is rubbed up in saline solution and filtered free of organisms; adding the culture-fluid, which has also been filtered so as to be germ-

free; and making the whole up to ten times the volume of the mass of tubercle bacilli and trichophyton used. The idea underlying this mode of preparation is that the trichophyton destroys some of the toxic bodies formed in the growth of the tubercle bacilli, while the immunizing principle remains unaffected. The result is a clear brown liquid, 1 c.c. of which contains the extract of 12.5 mg. of bacillary substance. The doses used are large, compared with other tuberculins, viz. 0.01, 0.1, 0.2 c.c. Rosenbach<sup>1</sup> advises the use of his tuberculin in all kinds of tuberculosis. It appears to be a very weak preparation, Lesser and Kogel<sup>2</sup> stating that it is 1,000 times weaker than Koch's O.T., while Schäfer<sup>3</sup> regards it as practically inert. Kausch<sup>4</sup> finds it useful in surgical tuberculosis, and Kohler and Plaut<sup>5</sup> in pulmonary disease.

#### TUBERCULOMUCIN

Weleminsky<sup>6</sup> grew cultures of tubercle bacilli for a prolonged period of time, and found that nucleo-protein, albumin, and finally mucin were formed in the process. After a time growth ceases, but the culture-fluid becomes fertile again on heating. This suggests that cessation of growth is due not to exhaustion of nutritive material, but to formation of an antibody. Weleminsky prepares a form of tuberculin from his cultures, the exact mode of preparation of which we have not found recorded. Pachner,<sup>7</sup> who tried it on 35 patients, found that it gave good results even in advanced cases. For use he dilutes the original fluid with saline solution, 1:10, and gives  $\frac{1}{10}$  c.c. of the dilution. The doses are doubled each time, injections being given weekly.

<sup>1</sup> *Deut. med. Woch.*, 1910, p. 1513.

<sup>2</sup> *Beitr. z. Klin. d. Tuberk.*, 1913.

<sup>3</sup> *Zeitschr. f. Tuberk.*, 1912, xii., Heft 2.

<sup>4</sup> *Deut. med. Woch.*, 1913, p. 252.

<sup>5</sup> *Zeitschr. f. klin. Med.*, 1912, lxxiv., Hefte 3 u. 4.

<sup>6</sup> *Berl. klin. Woch.*, 1912, p. 1321.

<sup>7</sup> *Beitr. z. Klin. d. Tuberk.*, 1912, xxv. 137.

## GROUP II

## TUBERCULOCIDIN AND ANTIPHTHISIN

It has been suggested that, in the process of preparing tuberculin usually adopted, some of the constituents of the bacterial culture, which are volatile, are driven off by the heat. Tuberculin also contains some ingredients which are harmful rather than curative. Klebs therefore devised modifications of tuberculin, prepared without heating, to which he gave the names of "tuberculocidin" and "antiphthisin." The method of manufacturing the former is complicated, consisting in frequent precipitation, and solution of the precipitate, by alcohol and other reagents. Tuberculocidin is said to kill the bacilli when it is added to a culture of them *in vitro*.

Klebs<sup>1</sup> claims good results from the use of his preparation, and a large amount of antiphthisin is said to be used in America. The therapeutic dose of tuberculocidin is 1 cg. to start with, the fluid as sold being diluted before use (1 : 10). Antiphthisin is more concentrated, and half the above quantity is administered.

Tuberculocidin may also be administered by the mouth, and is said to act as well or even better by this channel. It may be applied locally to tubercular lesions, and forms an efficient dressing for ocular ulcers, tuberculides, etc. Disease of bones and joints also improves under treatment with this remedy; in cases of tubercular hip-disease it should be injected in the neighbourhood of the joint. Tuberculocidin is said to have a favourable influence in cases of genito-urinary tuberculosis.

Jessen<sup>2</sup> states that the remedy is not free from danger. He gives doses of 20 to 25 drops, finding that larger doses may produce a reaction like that seen with the old tuberculin. He reports good effects from the use of tuberculocidin

<sup>1</sup> *Münch. med. Woch.*, 1904, p. 1688.

<sup>2</sup> *Centralbl. f. inn. Med.*, 1902, No. 23, p. 585.

in phthisis—fall of temperature, diminution in the amount of sputum, and feeling of improvement. All his cases in the first stage of the disease showed improvement (100 per cent.), and 54 per cent. of those in the second stage. Jessen thinks the remedy worthy of further trial.

There does not seem to be sufficient evidence available as to the curative effects of these preparations, which are now seldom if ever prescribed. Klebs claims 60 per cent. of cures among his cases, which does not seem a startling figure.

#### DENYS' TUBERCULIN (BOUILLON FILTRÉ)

This is prepared in a manner similar to Koch's T., but without the use of heat. It is sent out in eight different strengths, named respectively

$$\frac{\text{To}}{10,000}, \frac{\text{To}}{1,000}, \frac{\text{To}}{100}, \frac{\text{To}}{10}, \text{To}, \text{Ti}, \text{Tii}, \text{Tiii},$$

each being ten times the strength of that preceding it, so that a gradual increase of dose is facilitated.<sup>1</sup>

#### TUBERCULOL (TL.)

Landmann<sup>2</sup> devised this form of tuberculin with a view to avoiding the use of heat, which may destroy some of the immunizing substances of tuberculin when it is made according to Koch's formula. He took cultures of human bacilli made virulent by passage through animals, and extracted them with distilled water, first at room temperature, for a considerable time. The supernatant fluid was then decanted, and the bacilli macerated again with successive fresh quantities of water at temperatures of 60°, 80°, and 100° C., the liquid being each time similarly decanted. The materials thus obtained were mixed together, concentrated *in vacuo*, and filtered through porcelain, and the resulting sterile fluid constitutes Tuberculol B. The fluid in which

<sup>1</sup> *Bull. R. Acad. Méd. Belg.*, xvi. 499; see also Sahli, "Ueber Tuberkulinbehandlung," 1907.

<sup>2</sup> *Hygien. Rundschau*, 1900, x. 361.

the residue of the bacilli remained suspended was next filtered from the solid mass and constitutes Tuberculol C, while a mixture of this with the previously described Tuberculol B constitutes Tuberculol A. The preparation is standardized so that 1 c.c. contains the minimum lethal dose for a healthy guineapig. Von Holten,<sup>1</sup> who recommends this form of tuberculin, starts with a small dose, such as  $\frac{1}{200,000}$  c.c., of Tuberculol B, which he prefers to the other brands.

#### BERANECK'S TUBERCULIN

Beraneck<sup>2</sup> has prepared a form of tuberculin (T.B.K.) which he considers to be less toxic than Koch's. He obtains a toxin (*basitoxin*, B.T.) by growing the bacilli in veal-broth rendered slightly alkaline with calcium hydrate, at a temperature of 37° to 38° C., and evaporating the fluid *in vacuo* without heating. This is mixed with an equal quantity of a second toxin (*acidotoxin*, A.T.) extracted from the bodies of the bacilli. The preparation is sent out in a series of different dilutions,

$$\frac{A}{32}, \frac{A}{16}, \frac{A}{8}, \frac{A}{4}, \frac{A}{2}, A, B, C, D, E, F, G, H,$$

the last being the pure tuberculin, and each of the former brands being half the strength of that following it.

Sahli<sup>3</sup> has used the preparation with good effect, and finds the series of dilutions convenient. He starts with

$\frac{1}{20}$  c.c. of the dilution  $\frac{A}{32}$ , and increases the dose, if no result ensues, by successive amounts of  $\frac{1}{20}$  c.c.; he then uses the stronger solutions successively. The injections are given twice a week, or less frequently.

Good results in cases of pulmonary tuberculosis were also obtained by Paris,<sup>4</sup> who employed slightly different solutions.

<sup>1</sup> *Beitr. z. Klin. d. Tuberk.*, 1912, Suppl. IV., p. 106 (188).

<sup>2</sup> See *Semaine Méd.*, 1903, p. 393.

<sup>3</sup> *Op. supra cit.*

<sup>4</sup> *Rev. Méd. de la Suisse Romande*, 1904, No. 10, p. 629.

## GROUP III

## OXYTUBERCULIN

Hirschfelder<sup>1</sup> believed that toxins were converted into antitoxins by a process of oxidation, and consequently prepared a modification of tuberculin, which he called "oxy-tuberculin," by treating the ordinary substance with hydrogen peroxide. The hypothesis upon which the procedure is founded seems quite unsupported by evidence, and no records of the employment of the remedy are available.

## TUBERCULOTOXOIDIN

Ishigama<sup>2</sup> gave this name to a solution of tubercle bacilli in sulphuric acid, which he found to have some immunizing value and to raise the opsonic index. He also makes use of a serum prepared from animals immunized with this substance ("tuberculo-toxoidin immune serum").

## TUBERCULIN CL. (CALMETTE)

Calmette prepares a special form of tuberculin, which was originally intended for the ophthalmic test, but which has since been used for treatment. Broth-cultures, six weeks old, of bovine tubercle bacilli, are heated in the autoclave at 110° C. for twenty minutes. They are then evaporated on a water-bath at 80° C. to one-tenth of their original volume, and filtered. The filtrate is precipitated with 95 per cent. alcohol, and dried *in vacuo*; it is then dissolved again in water, once more precipitated with alcohol, and dried. The resulting powder is dissolved in sterile water for use, and is injected subcutaneously in graduated doses, from 0.001 mg. to 1 mg., at intervals of twelve days.

<sup>1</sup> *Deut. med. Woch.*, 1897; *Therap. Beilage*, 25.

<sup>2</sup> Ishigama and Matsuda, Abstr. in *Centralbl. f. Bakt.*, I. Ref., 1908, xli. 100.



## IODIZED TUBERCULIN

Bauer and Murschhausen,<sup>1</sup> and also Kapsenberg,<sup>2</sup> have prepared a combination of tuberculin with iodine. Kapsenberg's method is to take the mass of bacilli left in the preparation of tuberculin, and to rub it up with chloroform in a mortar. After the addition of more chloroform the liquid is centrifuged, when it divides into three layers, the bacilli remaining in the middle one. The top layer is a yellowish liquid which readily takes up iodine, 1 c.c. absorbing 5-10 drops of a saturated solution in 96 per cent. alcohol. The tuberculin thus formed is said to protect guineapigs against infection.

## IRON-TUBERCULIN

Schultz<sup>3</sup> devised a preparation derived from Old Tuberculin by the addition of 12 per cent. solution of iron oxychloride to a dilute watery solution (10 c.c. tuberculin with 50 c.c. water) of Koch's preparation. A precipitate forms, which is collected and dissolved in 1 per cent. caustic-soda solution. A sufficient quantity of 25 per cent. glycerin solution is added to prevent decomposition. The result is a clear, brownish, opalescent liquid, the dose of which is 0.01 mg., rising by multiples of 1.3, 1.7, 2.2, 3.5, 4.5, 6, 8, 10. Schellenberg<sup>4</sup> reports favourably of this iron-tuberculin (*Eisentuberkulin*), which can be used both for diagnosis and treatment. It is much weaker than O.T., resembling in strength albumose-free tuberculin, and can be safely used in out-patient practice.

## SANOCALCIN TUBERCULIN

On the ground that, experimentally, calcium salts aid phagocytosis (Hamburger and Hekma<sup>5</sup>), Sticker devised a

<sup>1</sup> *Beitr. z. Klin. d. Tuberk.*, Suppl. iii., 1912, p. 13.

<sup>2</sup> *Berl. klin. Woch.*, 1912, p. 897.

<sup>3</sup> *Ibid.*, 1909, p. 1721.

<sup>4</sup> *Zeitschr. f. Tuberk.*, 1912, xviii, 132.

<sup>5</sup> *Biochem. Zeitschr.*, 1907, No. 3

preparation combining this element with tuberculin. The composition of the preparation is—0.0005 gm. tuberculin and 0.01 calcium glycono-lacto-phosphate in 1 c.c. of sterile saline solution. It is sold in ampullæ, and the dose for starting treatment is 0.00005–0.0001 gm. tuberculin, subsequent doses rising by multiples of 2, and being given every two or three days. Korb<sup>1</sup> states that the preparation produces no febrile reaction or other ill effects, but a marked focal reaction in pulmonary disease. He believes it to be a useful remedy.

#### TEBEAN

This form of tuberculin, devised by Levy,<sup>2</sup> is made by shaking up human tubercle bacilli with 25 per cent. solution of galactose for four and a half days at 37° C. The resulting material is then dried *in vacuo*, and standardized so that 1 gm. of the powder contains 1 mg. of dried bacillary substance. It is said to have some immunizing power in guineapigs, those injected with tebean living longer than control animals, and showing at times cavity-formation in the lungs, a sign of chronic disease due to increased resistance. For therapeutic use the powder is dissolved in sterile saline solution, the doses rising from  $\frac{1}{100}$  mg. to 4 mg. and being given once or twice weekly. Steffen<sup>3</sup> records good results from the use of this preparation.

#### SERUM DIAGNOSIS

**Agglutination test for tuberculosis.**—It is found that the blood of a patient suffering from tuberculosis has the power of causing a clumping of tubercle bacilli in the same way as that of a typhoid patient agglutinates the *B. typhosus*. Use was made of this property by Arloing and Courmont<sup>4</sup> to detect the presence of tuberculosis.

<sup>1</sup> *Zeitschr. f. Tuberk.*, 1913, x'x, 339.

<sup>2</sup> Levy and Krencker, *Zeitschr. f. Immunitätsforsch.*, Orig., 1909, iv, 286.

<sup>3</sup> *Münch. med. Woch.*, April 19, 1910, p. 838.

<sup>4</sup> *Gaz. des Hôp.*, 1900, p. 1467.

**Preparation of the emulsion.**—In the case of the tubercle bacillus a preliminary difficulty arises, which is not found in working with the *B. typhosus*, in that the former organism, when grown in the laboratory on ordinary culture-media, occurs in masses which are already closely agglutinated. It is necessary, therefore, for the purpose of the test, to prepare the bacilli in some special way so that they are separated one from another. This was first accomplished by Arloing<sup>1</sup> by the following method of procedure: Suitable potatoes are taken and boiled, and slices of them are put into the usual laboratory potato-tubes. At the bottom of the tubes is placed a small quantity of a 6 per cent. solution of glycerin in water, so that the fluid just touches the lower part of the potato. The tubes thus prepared are sterilized for forty-five minutes in the autoclave. The surfaces of the slices of potato are then inoculated with tubercle bacilli derived from a human source, and the cultures are incubated at a temperature of 38° to 39° C. On every second day the tubes are tipped up, so that by their inclination the glycerin solution is caused to flow over the cultures on the surface of the potato. Growth occurs rapidly in these circumstances, the resulting masses of organisms being different from the ordinary cultures on glycerin-agar, in that they are soft in consistency, and easily broken up by the application of a glass rod, or by rubbing in a mortar. From these cultures sub-cultures are made in glycerinated veal-broth (1 per cent. peptone and 6 per cent. glycerin). These are submitted to daily shaking to keep the organisms separate one from another. Even in these cultures it is impossible to prevent a certain amount of clump-formation, but the majority of the organisms present are separate, and the fluid is turbid and fairly homogeneous, with but little sediment.

It is interesting to note that, grown under the conditions indicated, the bacilli are described as motile, some writers even attributing to them a degree of motility

<sup>1</sup> *Compt. Rend. Acad. Sci.*, 1898, cxxvi. 1319.

equal to that of the *Bacillus typhosus*. Branching forms are also met with. Koch at one time doubted the identity of the bacilli described by Arloing and Courmont with his own bacilli.

Some writers advise that, before they are used for the agglutination test, the organisms should be transferred through a series of broth-cultures, holding that they thus become more motile, are better separated, and grow more rapidly. Loeb<sup>1</sup> does not recommend this procedure, as he has found that it is impossible to grow the bacilli beyond the fourth generation. This observer has failed to discover active movement in the bacilli, though the usual Brownian movement may be seen.

The broth-culture should be grown for a period of from nine to fifteen days, and then used for the test. Before the eighth day there are not enough bacilli present, while after the fifteenth, spontaneous agglutination may take place, and the reaction with serum is often lessened at this time. The test-fluid can be preserved in a condition fit for use by keeping it on ice, or by the addition of a minute quantity of some antiseptic, e.g. formalin (1 : 400), or carbolic acid; both methods may be combined. Some such mode of preservation is necessary, as the labour of making a separate culture for every experiment would be enormous.

**Mode of performing the test.**—The mode of applying the test is as follows: Clear blood-serum or inflammatory fluid from a patient suspected of tuberculosis is added in varying proportions to a series of tubes of the suspended bacilli. The tubes are placed in an incubator for a period of two to six hours, inclined at an angle of 45°. If they are allowed to remain for a longer period, for instance twenty-four hours, as was at first recommended, normal serum may give rise to a certain amount of agglutination. If the reaction be positive, the serum gradually appears less opaque, a flocculent precipitate falling to the bottom. This is visible with the unaided eye, on examination in a bright

<sup>1</sup> *Journ. of Amer. Med. Assoc.*, May 23, 1903, pp. 1, 423, etc.

light against a dark background. A control experiment should be made, for purpose of comparison, with normal serum. Microscopically, it will be seen that the bacilli are clumped as in the "Widal test" for enteric fever. The test in tuberculosis is not, however, so well marked as in the former disease. Errors may occur owing to the presence of small fibrinous coagula, especially when inflammatory exudates are employed for the test. It is necessary to make certain by means of the microscope that any apparent clumps are in reality formed of bacilli. Staining reagents may be needed to decide in cases of doubt. It must be borne in mind that a certain proportion of clumped bacilli may occur in the cultures, however carefully they are prepared; hence arises the importance of invariably making use of a control experiment.

**Simplification of the procedure.** — The process adopted by Arloing and Courmont is very long and tedious, and modifications have been suggested for the purpose of simplifying it. Thus, Romberg prepared a suspension of the bacilli by macerating dried cultures with a 1·5 per cent. solution of caustic soda, and then neutralizing with acetic acid. Koch<sup>1</sup> described a still simpler method. An ordinary culture is taken and dried by pressure between pieces of blotting-paper. A known quantity of it is then weighed out and rubbed up in a mortar with a weak solution of caustic soda. Instead of this a culture may be dried and triturated in a mortar to a fine powder. A weighed quantity of the powder (0·1 gr.) is macerated with saline solution, added in quantities of a few drops at a time, till the original culture is diluted to 1 : 100. The solid residue is then separated by the centrifuge, and the supernatant fluid is decanted and diluted with a fresh amount of salt-solution, to which a small amount of carbolic acid is added, till the dilution reaches 1 : 1,000. This fluid can be kept without alteration owing to the presence of the carbolic acid. For use it is again diluted to 1 : 10,000,

<sup>1</sup> *Deut. med. Woch.*, Nov. 28, 1901.

but this last dilution seems unnecessary and almost disadvantageous. If a strongly clumping serum is added to the fluid in the proportion of 1 : 10 or 1 : 25, agglutination rapidly occurs. This is aided by a moderate degree of warmth, as by holding the test-tube in the hand. The reaction takes place much more quickly in the stronger fluid (1 : 1,000) than in the extreme dilution. The time-limit recommended by Koch is fifteen to twenty hours. A good plan is to put the tubes in the incubator overnight, and to examine them in the morning. Koch employed dilutions of serum of 1 : 10, 1 : 25, 1 : 75, 1 : 100, and so on, in a series of tubes. The serum is first poured into the test-tube and the fluid containing the bacilli is added, and the mixture shaken up. A control test is always necessary.

Koch advised removal of the serum needed by means of a cupping-glass, while Arloing and Courmont draw blood from a vein and remove the corpuscles by the centrifuge. Shibayama<sup>1</sup> recommends for this test the use of organisms freed from fat by extraction with alcohol and ether, and made into a homogeneous emulsion.

**Agglutinative power in human beings.** — In human beings it is found that the serum of those who are not suffering from tuberculosis may at times possess an agglutinative power. Infants and young children do not seem to give a reaction, but adults may do so. Thus, of 30 non-tuberculous persons, 5 gave a reaction in dilutions of 1 : 25, 1 at 1 : 50. In one case a subsequent post-mortem examination proved the absence of tubercular infection. Of 78 phthisical cases, only 14 gave a positive reaction in dilutions of 1 : 10, 1 case at 1 : 50, 4 at 1 : 25. In several cases of tuberculosis affecting other regions (bladder, bone, etc.) no reaction was obtained.

Arloing and Courmont give the following statistics of results : Of 191 persons presenting clinical signs of tuberculosis, 168 or 87.9 per cent. reacted positively, while 23 or 12.1 per cent. were negative. Of 130 cases clinically non-

<sup>1</sup> *Berl. klin. Woch.*, 1911, p. 341.

tuberculous, 45 reacted (34·6 per cent.), 85 (65·4 per cent.) were negative. Among 41 healthy persons, 11 reacted (26·8 per cent.), while 30 were negative (73·2 per cent.). In all these cases blood-serum was employed for the test. Serous effusions gave the following results :—

			Positive		Negative
Tubercular pleural effusions	(31)	...	23	...	8
Pleurisy of doubtful origin	(16)	...	13	...	3
Non-tubercular hydrothorax	(11)	...	0	...	11
Tubercular ascites ...	(13)	...	11	...	2
Non-tubercular ascites ...	(20)	...	0	...	20

In cases of tubercular meningitis the result was always negative in children, but two adults gave positive reactions. The above figures would suggest that the test may be a valuable aid in the diagnosis of tubercular peritonitis from conditions which produce similar symptoms, such as cirrhosis of the liver and chronic simple peritonitis, if the latter condition really exist. The failure of the reaction in 12 per cent. of clinically tuberculous cases suggests that for ordinary use the test is of doubtful value. The non-appearance of the reaction in cases of tubercular meningitis in children is particularly unfortunate, as this disease is a very insidious one, for which a sure test would be of the greatest service, while it would certainly not be legitimate to make use of tuberculin in such a malady.

The results obtained by Beck and Rabinovitch<sup>1</sup> were very much less favourable for the value of the test. Thus, in cattle, among 19 healthy beasts, 12 gave a positive reaction, and among 4 suffering from diseases other than tuberculosis, 3 reacted. Among 17 beasts with early tubercle, 6 were negative, and among 22 moderately advanced cases 2 were negative and 6 only reacted in a dilution of 1 : 5, at which point the serum of even healthy cattle may cause agglutination. Among 16 very advanced cases, 1 was negative and 4 reacted only at 1 : 5. In human beings

<sup>1</sup> Quoted by Loeb, *loc. cit.*



these observers record that among 17 cases of incipient tuberculosis only 6 gave a positive reaction, and among 16 advanced cases only 4 reacted. Among 5 doubtful cases which gave a positive reaction with tuberculin only 1 reacted positively with the agglutination test. On the other hand, of 31 non-tuberculous cases, 10 reacted positively.

Humbert<sup>1</sup> found the reaction positive in 4 cases of miliary tuberculosis, and thinks the test useful; as do also Sabarcanu and Salomon,<sup>2</sup> Vasilescu<sup>3</sup> (diagnosis from enteric fever, influenza), and Grysez and Job.<sup>4</sup> Friedmann<sup>5</sup> and Kington and Twichell,<sup>6</sup> however, find it of no value. Simoni<sup>7</sup> found it of use in the diagnosis of tubercular ear-disease, and Pellegrini,<sup>8</sup> who admits that it is uncertain, in surgical tuberculosis.

On the whole, it seems necessary to conclude, on the evidence at present available, that the agglutination test is of little or no practical use in the diagnosis of tuberculosis. This is the opinion of Koch and of Beck and Rabinovitch. The margin of error is too great for the test to afford trustworthy indications for clinical use. The most hopeful field for further experiments with this reaction is in the diagnosis of tubercular ascitic effusions, in which the fluid is easily obtained, and in which, so far, the recorded results are encouraging.

**Complement-fixation.**—The application of this reaction as a test for the presence of tuberculosis has been the subject of many observations recently, various antigens being tried by different experimenters, such as Old and New Tuberculin, defatted tubercle bacilli (Momose), and extracts of tuberculous organs (Hammer). Early observations were

<sup>1</sup> *Rev. de la Tuberc.*, 1904, p. 233.

<sup>2</sup> *Rev. de Méd.*, 1905, No. 7.

<sup>3</sup> *Inaug. Diss. Bucharest*, 1905.

<sup>4</sup> *Rev. de Méd.*, 1906, p. 705.

<sup>5</sup> *Abstr. in Centralbl. f. inn. Med.*, 1906, p. 757.

<sup>6</sup> *Amer. Journ. Med. Sci.*, Oct., 1906.

<sup>7</sup> *Gaz. degli Ospedali*, May 1, 1904.

<sup>8</sup> *La Clin. Mod.*, 1904, x., Nos. 27, 28.

made by Citron and by Nesfield. Wwedensky,<sup>1</sup> who tried the reaction in cases of surgical tuberculosis, found that it was not of much use in the diagnosis of early cases, and might fail in cases which were clinically obvious; as a rule, early active tubercle and miliary infection gave positive results, while old chronic cases were frequently negative. Probably surgical (localized) cases are less favourable for the formation of antibodies than those of pulmonary disease, in which absorption of toxins is theoretically more constant and considerable.

Dudgeon, Meek and Weir,<sup>2</sup> who used rubbed-up bacilli as antigen, found a positive reaction in 86 of 100 clinically tuberculous patients who had not been treated with tuberculin. After such treatment a positive reaction was invariably obtained.

Besredka<sup>3</sup> prepared a special antigen by cultivating separately human and bovine bacilli on a medium consisting of 5 parts broth, 4 of albumin, and 1 of yolk of egg (or alternatively 10 parts broth and 0.5 part egg-albumin) for thirty days. The cultures are sterilized and mixed. Working with other helpers, he found that in guineapigs the reaction becomes positive four days after infection when there are no visible anatomical lesions; it disappears as the disease spreads, but afterwards reappears when the infection is widespread, and persists till shortly before the death of the animal. In rabbits, the reaction is strongest in animals which have considerable resistance to the bacilli. In man, out of 750 persons taken at random, 665 were negative, 16 doubtful, and 69 positive. Of these last, 53 were clinically tuberculous. Of 107 definitely tuberculous patients, all in the early stage gave positive reactions, and most of those in the middle period of the disease. Advanced cases reacted

<sup>1</sup> *Rousski Vrach*, 1913, p. 1540: abstr. in *Zeits. f. Immunitätsforsch.*, Ref., 1913, p. 931.

<sup>2</sup> *Lancet*, 1913, i. 19.

<sup>3</sup> *Compt. Rend. Acad. Sciences*, 1913, p. 1633; abstr. in *Zeits. f. Immunitätsforsch.*, I. Theil, 1914, xxi. 77.

irregularly, some giving positive, others negative reactions. Of 43 clinically non-tuberculous patients, all gave a negative reaction. Inman,<sup>1</sup> working at Brompton Hospital with Besredka's antigen, found that out of a first series of 52 tuberculous patients, 50 gave a positive reaction, 2 a negative. In a series of 107 cases which we had the opportunity of observing clinically the results were as follow :—

<i>Clinical diagnosis</i>	<i>No. of cases</i>	<i>Complement-fixation test</i>	
		<i>Positive</i>	<i>Negative</i>
Clinically tuberculous .	70	67 (95·6 %)	3
Clinically doubtful . . . . .	11	4	7
Clinically non-tuberculous . . . . .	22	4 doubtful	18 (81·8 %)
Tuberculosis, not pulmonary . . . . .	2	2	—
Apical fibrosis in children . . . . .	2	1 doubtful	1
Total . . . . .	107	78	29

Much less satisfactory results were obtained with an antigen prepared in the hospital laboratory.

Marmorek<sup>2</sup> devised a complement-fixation technique carried out with his antitubercular serum and with either the serum or the urine of tuberculous patients. The procedure consists in mixing 0·2 c.c. of the patient's serum or a similar amount of urine with 0·3 c.c. of Marmorek's serum and 0·5 c.c. of fresh guineapig's serum (complement), and incubating for one hour. Sensitized erythrocytes are then added, and the mixture again incubated for three-quarters of an hour. If there is no hæmolysis, the reaction is positive. Marmorek, who tested 306 specimens of serum and 294 of urine, found that in only 28 cases (5 per cent.) did the test fail to correspond with the clinical diagnosis. Bergeron<sup>3</sup> found that of 72 cases of febrile tuberculosis

<sup>1</sup> *Lancet*, 1914, i. 1446. ‡

<sup>2</sup> *Presse Méd.*, 1909, No. 12.

<sup>3</sup> *Ibid.*, 1910, No. 1.

63 reacted strongly (no hæmolysis) and 7 weakly (partial hæmolysis), while 2 were doubtful (traces of hæmolysis). Of 14 cases of tuberculosis of the serous membranes, 7 reacted strongly and 7 weakly. In slight or apyrexial tuberculosis the results were not good, only 23 cases out of 42 giving a positive reaction. Klinkert<sup>1</sup> holds that the reaction is not sufficiently specific to be of service in diagnosis.

McIntosh, Fildes and Radcliffe,<sup>2</sup> using a suspension of tubercle bacilli in saline fluid as antigen, record the following results :—

				Cases	Positive	Negative
(a)	Pathologically certain tuberculosis—					
	Pulmonary	...	...	43	33 (76·7 %)	10
	Surgical	...	...	26	21 (80·7 %)	5
	Glandular	...	...	16	6 (37·5 %)	10
(b)	Clinically certain tuberculosis—					
	Pulmonary or pleural	...	...	18	10 (55·5 %)	8
	Surgical	...	...	42	30 (71·4 %)	12
(c)	Doubtful tuberculosis (various)				18 (24·4 %)	56
(d)	Controls	...	...	87	33 (3·4 %)	84

They conclude that a positive reaction definitely indicates active tuberculosis, and that the test is thus more valuable than the cutaneous tuberculin test of von Pirquet.

**Precipitation test.**—Porter<sup>4</sup> described a precipitation test for tuberculosis which is carried out as follows: To 1 c.c. of B.E. are added 19 c.c. of sterile distilled water, and the mixture is kept at 37° C. for twenty-four hours. It is then divided into two portions: to one (A) is added water up to 25 c.c., with enough sodium chloride to make the whole isotonic; to the other (B), the same fluid with the addition of phenol to make 0·5 per cent. These mixtures are kept for

<sup>1</sup> *Zeitschr. f. exper. Pathol.*, 1911, viii. 451.

<sup>2</sup> *Lancet*, 1914, ii. 485.

<sup>3</sup> Two cases of leprosy and one of Addison's disease. The serum in 18 syphilitic cases was uniformly negative.

<sup>4</sup> *Journ. Infect. Dis.*, 1910, vii. No. 6.

twelve hours at 37° C. and then filtered through porcelain. The serum to be tested is diluted to 1 : 20 with 0·5 per cent. saline solution, and divided into three tubes. To one is added an equal quantity of solution (A), to a second the same amount of solution (B), and to the third the same amount of phenolized (0·5 per cent.) saline solution. These mixtures are incubated at 37° C. for twenty-four hours, and then examined for the formation of a precipitate, the last-named mixture being the control. Porter finds 40 per cent. of early cases, 62 per cent. of chronic cases, and 20 per cent. of advanced and acute cases positive, against about 12 per cent. positive in non-tuberculous cases. The method does not seem to be of much value as a means of diagnosis.

## DIAGNOSTIC USE OF TUBERCULIN

*Veterinary use.*—As a means of recognizing the presence of tuberculosis, tuberculin has proved of the greatest service, especially in veterinary practice. It is of considerable importance to be able to discover the existence of the disease in herds of cattle; and by injecting the animals with Koch's preparation the diagnosis can be made with almost entire certainty, even in the absence of any symptoms of the malady. The injection does no harm to the beasts beyond the temporary febrile symptoms which it produces in those which are tuberculous. No effects at all are produced in healthy animals. Thus McEachran<sup>1</sup> records the use of tuberculin in 22,023 cases in cattle without any ill effects.

**Employment in man.**—In mankind the use of tuberculin as a diagnostic agent has been much debated. It must be remembered that the results of careful post-mortem examinations have proved that tubercular infection is very widely spread among all classes of the population, so that some 80 per cent. of all persons who reach the age of 40 probably have healed tuberculous lesions in some part of the body, this percentage falling in lower age-groups, but

<sup>1</sup> *Trans. Brit. Congr. of Tuberc.*, iv. 114.

being still very considerable in all individuals above 5 or 6 years of age. Infected persons, who have overcome the infection and are apparently quite healthy, may yet give a positive reaction to tuberculin. Hence the value of any form of tuberculin test is greatest in children and diminishes in value with advance in age.

Where a hypodermic injection of tuberculin is administered for diagnostic purposes, two phenomena are of importance besides the rise of temperature and constitutional disturbance that follow in tuberculous subjects. There is in such persons often considerable *local* reaction at the seat of infection, in the form of redness and œdema, which may appear apart from febrile disturbance and yet point to some tubercular infection, probably of an inactive kind if the local effects alone are observed. There is also in some cases a *foral* reaction at the seat of the tuberculous lesion which is of particular importance as indicating the seat of the tuberculous lesion. Thus in a consumptive person fresh physical signs (*râles*) may appear in the chest as the result of an injection of tuberculin, definitely pointing to the lungs as the seat of disease; in cases of tuberculous joints there may be pain and signs of fresh arthritis; in genito-urinary tuberculosis, hæmaturia; and so forth. In pulmonary cases this local exacerbation may lead to the appearance of tubercle bacilli in the sputum, which should be examined afresh after giving an injection.

That tuberculin is of service diagnostically cannot be denied, but it has been held that there are *drawbacks* which counterbalance its usefulness.

(1) It is urged that in a certain proportion of cases the injection of tuberculin may light up again an infection which has become quiescent, and may thus cause an exacerbation of the disease. It is very difficult to make certain of the facts in this respect, since tuberculosis is a disease which is very liable to sudden exacerbations without the administration of any drug, and it is probable that

many of the ill effects attributed to the action of tuberculin have been only accidental concomitants.

Koch, writing in 1897, remarked: "The most valuable property of tuberculin is that, even when injected subcutaneously in very minute doses, it gives rise to the characteristic reaction in both men and animals affected with tuberculosis. The value of tuberculin as a diagnostic agent, on which I laid stress in my first publication on tuberculin, has been more and more fully vindicated with the lapse of time. The fear that along with the reaction tubercle bacilli might be set free and gain a footing in healthy parts of the body has been proved to be unfounded in many thousands of injections into cattle made for the purpose mentioned. In not one single case was it possible to detect any indication of such unfettering of the bacilli. In view of this evidence the foolish prejudice resting on the supposed setting-free of the bacilli should be abandoned, and use should be made of the diagnostic properties of tuberculin." At the British Congress of Tuberculosis, Koch quoted 3,000 tests made with tuberculin in man without any ill effects; and Anders<sup>1</sup> alludes to 3,638 similar injections which were equally harmless. On the other hand, Munzer<sup>2</sup> and Behring<sup>3</sup> regard the injections as distinctly dangerous; and we believe that, if large doses are given, there is solid ground for such fears.

(2) There is some danger of actually inoculating living and virulent tubercle bacilli in the tuberculin. This can hardly be the case with the old tuberculin, which is generally used for diagnostic purposes; but in the new tuberculin Thellung<sup>4</sup> found virulent bacilli, and actually produced infection in rabbits and guineapigs.

(3) The test cannot be used in cases in which the patient's

<sup>1</sup> *Trans. Amer. Climatol. Assoc.*, 1900.

<sup>2</sup> *Prag. med. Woch.*, 1903, March, No. 13, p. 145.

<sup>3</sup> *Gesellsch. f. inn. Med. Wien.*, March 12, 1903.

<sup>4</sup> *Deut. med. Woch.*, 1901, No. 48; and *Centralbl. f. Bakt.*, I. Orig. 1902, xxxii. 28.



temperature rises (apart from the use of tuberculin) to as high a point as 100° F., because in such instances it is not possible to make sure of the reaction. It is also apparently unwise to use the drug in febrile cases, as they may be injuriously affected by it.

(4) Most important of all as a drawback to the use of the test for diagnosis is the fact, now ascertained, that the reaction is obtained not only in cases of active tuberculosis, but also in old quiescent cases (and it is in these that there appears to be some danger of lighting up the disease afresh), and in some persons suffering from entirely different complaints. It has certainly been demonstrated that the test is not so absolutely infallible as was at first expected. Thus Madison<sup>1</sup> finds that there may be marked reaction to tuberculin in cases in which, post mortem, no sign of tuberculosis can be found. He also quotes cases of healed tubercle which gave a reaction with the test; while he has met with patients suffering from undoubted tuberculosis who were unaffected by the injections. He places the margin of error at 10 per cent. K. Franz<sup>2</sup> (who considers that there is no danger in the injections) found that the presence of a reaction in healthy persons was very rare, but that in those who are out of health, especially in individuals who are the subjects of syphilis, a reaction to tuberculin is liable to occur. He made experiments on a number of recruits, and considers that on the whole the test is useful and reliable.

At the London Congress of Tuberculosis, E. France related the results which he obtained upon a number of insane patients. Out of 55 persons tested he found that 45 reacted to tuberculin. Twenty-nine of the latter died, and were submitted to necropsy. All of these 29 were proved to be suffering from tuberculosis at the time of death. Among those who did not react to the injections 5 died, and were examined after death; none of these were found

<sup>1</sup> *Amer. Med.*, Dec. 20, 1902.

<sup>2</sup> *Wien. med. Woch.*, 1902, Nos. 36-38.

to be tuberculous. These results are very favourable to the use of tuberculin.

Koch himself claimed 99 per cent. of correct results from the use of the test. This can hardly be maintained in view of the results of other observers, unless we ascribe special skill to the inventor of the test. Probably the estimate of 10 per cent. of error is not far wrong.

The result of a test with tuberculin may be inconclusive in individual cases in which the question of the tubercular or non-tubercular nature of a particular lesion is at issue. As an instance the following case may be quoted: The present writers administered 0·01 c.c. to a weakly child of 5, weighing only  $1\frac{1}{2}$  stone, who was suffering from enlarged joints—with a view to determine the nature of this trouble. A reaction ensued, consisting in a rise of temperature (which had previously been normal) to  $103^{\circ}$  F., with rather troublesome vomiting. The little patient did not seem to feel ill, but complained a good deal of the sickness, as she could not keep her food down although she felt hungry. No ill effects ensued, beyond a slight degree of redness and induration at the point of injection, which appeared about the third day, and passed off by the fifth or sixth. The temperature fell by lysis, rising on the evenings of the three ensuing days, but each time to a lower figure than on the previous night. No signs of redness or swelling were seen in the neighbourhood of the joints, as should have occurred had the lesions been tubercular: yet the febrile reaction had been marked. This may, however, have been due to some small focus of tuberculosis in the lungs or elsewhere.

Tinker<sup>1</sup> states that if the dose be large enough, even healthy persons react. He also points out that different specimens of tuberculin vary much in strength, and that a source of error thus arises in comparing results obtained. He lays stress on the advisability of beginning with small doses.

In view of the unpleasantness of the results of the

<sup>1</sup> *Johns Hopkins Hosp. Repts.*, 1903, xi, 544.

injections (fever, vomiting, etc.) in many patients, as well as the possibility of exciting an exacerbation of the disease—however remote this possibility may be—we should refrain from making use of this means of diagnosis unless there exist special reasons for its employment.

Definite **contraindications** to the use of diagnostic doses are the existence of fever, nephritis, hæmoptysis, and cardiac failure. Epilepsy is also considered by Bandelier and Roepke<sup>1</sup> to constitute a sufficient danger to make the procedure unadvisable, as are also diabetes, arterio-sclerosis, and amyloid change. It should not be used upon very debilitated persons or during menstruation; while in hysterical patients a rise of temperature after an injection is not of the same diagnostic import as in less emotional subjects. Used with these limitations, and with due regard to the margin of error alluded to, there can be no doubt that we have in tuberculin a valuable assistance in the diagnosis of early or obscure cases of tuberculosis.

**Mode of using tuberculin for diagnostic purposes.**—Old tuberculin is generally supplied in small glass bottles containing 1 c.c. For use it must be diluted with a 25-per-cent. solution of glycerin, if small doses are needed. Thus, to administer 0.01 c.c. the quantity contained in the original bottle may be diluted with 9 c.c. of glycerin solution, and  $\frac{1}{10}$  c.c. of the resulting fluid given hypodermically. The position for the injection is immaterial. Slight redness and œdema may occur at the point of injection, but this passes off without any ill effects.

The advice given by Koch for the diagnostic use of tuberculin is as follows: It is necessary to observe the course of the patient's temperature carefully for a day or two—preferably two—before the injection is given, in order to make sure that the daily excursion is within moderate limits. A temperature of 100° F. is a contraindication to the use of tuberculin, as not only does the existence

<sup>1</sup> "Lehrb. der spec. Diagnostik u. Therap. der Tuberk." Würzburg, 1908.

of such a degree of fever render it difficult to ascertain the exact effect produced by the injection, but the condition of such febrile cases is sometimes depressed by the remedy. If the patient is suitable in the above respect, it is necessary to take into account also his general state of strength or weakness, in order that the dose of tuberculin may be modified accordingly. Delicate individuals receive for a first injection 0·0001 c.c., whereas those who appear to be in fair health may at once receive 0·001 c.c. The injection is given beneath the skin of the back between the scapulæ. The reaction may be expected in about twelve hours, and Koch prefers to give the injection in the afternoon. If no reaction takes place, a second dose of double the quantity first administered is given on the third day; while if a very slight reaction, such as a rise of half a degree, occurs, the same dose as that which produced this effect is repeated. A much more marked rise of temperature is often seen after this procedure. Koch regards this phenomenon (increased reaction on repetition of a small dose) as very characteristic of tuberculosis. If, however, no effect is produced by the small doses, they may be increased to 0·5 and even to 1·0 c.c.; and this final dose may be administered twice in order to make sure of the absence of a reaction.

Junker<sup>1</sup> advises the following doses for diagnostic purposes: 0·01, 0·05, 0·1, and 0·5 c.c. Lowenstein and Kaufmann<sup>2</sup> give 4 doses of 0·02 c.c., and if there is no reaction continue with 0·2, 0·5, and 1·0 c.c. Roepke<sup>3</sup> finds this repetition of 0·02 c.c. insufficient in many cases; he advises successive doses of 0·02, 0·1, and 0·5 c.c.

In the case of children, Escherich gives 0·02 to 0·05 c.c. to younger, and 0·05 to 0·1 c.c. to older patients; Beck gives 0·05 c.c. to all under 10 years; Leser gives children  $\frac{1}{5}$  to  $\frac{1}{2}$ , and Heubner  $\frac{1}{20}$  of the adult dose; Epstein

<sup>1</sup> *Beitr. z. Klin. der Tuberk.*, Bd. vi., Heft 4.

<sup>2</sup> *Zeitschr. f. Tuberk.*, 1907, Bd. x. 17.

<sup>3</sup> *Ibid.*, 1907, x. 5.

gives children under 3 years 0·01 c.c., and gradually raises the dose by quantities of 0·005 or 0·01 c.c.<sup>1</sup>

Trudeau gives the injection as late at night as possible, so as to bring the reaction to a convenient time of day. He insists on the importance of using a fresh tuberculin solution, which he prepares with  $\frac{1}{2}$ -per-cent. carbolic-acid solution; it must not be more than three days old. He starts with a dose of 0·001 c.c., and, if this produces no reaction, goes on to doses of 0·003 c.c. and then to 0·005 and 0·007. M. Beck begins with a dose of 0·001 c.c. even in weakly persons. In children under 5 years of age he starts with 0·0003 c.c., and goes on to a second dose of 0·001 c.c., and then to one of 0·005 c.c. In children between 5 and 10 he starts with 0·0005 c.c., and gives 0·005 as the maximum dose.

One-thousandth of a cubic centimetre is probably the largest dose which should ever be administered for diagnosis. The authors consider that doses of 0·0001 c.c., 0·0002 c.c., 0·0005 c.c., 0·001 c.c., form the most suitable series.

**Conclusions as to the diagnostic use of tuberculin.**—Taking all the evidence at present available, the conclusion appears to be that there is a certain degree of danger in administering a diagnostic injection of tuberculin, but probably not more than in giving an anæsthetic for similar purposes. In no case should we adopt either means if it is possible to make a diagnosis otherwise; but if the matter is one of urgency we should not hesitate to make use of the drug. In doubtful cases of phthisis, careful physical examination should be first made, and the sputum should be examined for tubercle bacilli. If these methods do not clear up the nature of the case, we must consider, from the point of view of the interests of the patient, whether it is necessary to resort to an injection of tuberculin. In the majority of instances probably it is preferable to wait, the patient being meanwhile put into the most favourable possible circumstances

<sup>1</sup> Quoted by Schick, *Jahrb. f. Kinderheilk.*, 1905, lxi. 811.

to combat the disease, if it be present. Open-air life and plentiful feeding will form suitable treatment for the majority of conditions which are liable to be confused with tuberculosis. But there are a certain number of cases in which the question of the presence or absence of tubercle is of such importance that any means of reaching certainty without further delay should be adopted. Such an instance might be seen in the case of a young man just starting in life, who had to decide on what profession or course of life he should adopt. It might be a question whether it was right for him to enter on an indoor life in a London office, or better that he should emigrate and lead an open-air existence in one of the colonies. Such a question might need an immediate answer, and an injection of tuberculin might here be not only permissible but advisable. So, too, might it be in the case of a young woman belonging to a tubercular family, who had perhaps recently suffered from pleurisy, and who sought advice as to the propriety of marrying. But such cases will constitute the minority of those met with in practice. The test should not be used indiscriminately, merely for our own satisfaction. The danger run may be minimal, but for such a purpose we have no right to run any danger at all. It is not justifiable to begin with large doses of the drug.

**Special modes of administering tuberculin for diagnosis.**—Von Pirquet<sup>1</sup> has suggested the inoculation of tuberculin into the skin instead of hypodermic injection. For this purpose the skin is cleaned as if for a surgical operation; a drop of tuberculin is placed upon it; and through this a series of scratches are made as in vaccination, or preferably a circular scarification made by means of a special "Von Pirquet" platinum spud with three sharp points. A "control" lesion is made by scratching with another aseptic needle through a drop of sterile saline solution at another point. In tuberculous subjects this procedure is followed by an inflammatory reaction at the seat

<sup>1</sup> *Deut. med. Woch.*, May 23, 1907.

of inoculation with the tuberculin (**cutaneous reaction**): this varies from slight redness round the scratches, to an indurated papule and even a vesicle or group of vesicles, which may subsequently undergo desquamation. The reaction usually occurs within twenty-four hours: it is occasionally delayed, and may be at its maximum on the third or fourth day. A certain amount of pigmentation may occur, and persist for several weeks. There is no danger in this procedure, but its value as a test for the presence of tuberculosis is disputed.

Ellermann and Erlandsen<sup>1</sup> modified this test by making four separate scarifications each through a drop of tuberculin varying in the degree of dilution—the strengths recommended being 1 per cent., 4 per cent., 16 per cent., and 64 per cent. (**quantitative cutaneous reaction**). In the non-tuberculous or papular form they are all more or less of equal size; in the tuberculous the size of the papules increases with the percentage of tuberculin employed. The scarifications are usually made on the forearm in its long axis, the 1-per-cent. drop being deposited nearest the hand, the 4-per-cent. drop about an inch higher up, and so on. The measurement of the resulting papules is carried out twenty-four and forty-eight hours later, when the sum of the average diameters is utilized to obtain an average *papule size*, which, together with the figure representing the average difference between successive papules (*papule difference*), is applied to an empirical table to ascertain the appropriate figure, which is termed the *sensitiveness value*. The authors regard 100 as the crucial figure and consider sensitiveness values above this as indicating active tuberculosis. In this modified form the cutaneous test appears to have considerable diagnostic value.

Moro<sup>2</sup> employs inunction of old tuberculin worked up with an equal bulk of lanolin to produce a similar reaction (**percutaneous reaction**).

<sup>1</sup> *Deut. med. Woch.*, 1909, xxxv. 436.

<sup>2</sup> *Münch. med. Woch.*, 1908, xlv. 209.



Calmette, of Lisle, and Wolff-Eisner, of Berlin, independently suggested the instillation of tuberculin into the conjunctival sac as a test for the presence of tuberculosis. The procedure is generally known as Calmette's **ophthalmic reaction**. It can be performed with a solution of old tuberculin (1-10 per cent., Wolff-Eisner); but owing to the irritant action of the glycerin contained in this preparation, a solution of the precipitate obtained by treating tuberculin with absolute alcohol is generally employed. This can be obtained commercially in a form suitable for making the solutions. A drop of the fluid is placed in the hollow formed by drawing down the lower eyelid, and the patient is directed to hold the head back for a minute or so, in order that the tuberculin may not at once escape. In tuberculous subjects an inflammatory reaction ensues within twelve to twenty-four hours. The untreated eye serves as a control for comparison. As soon as the reaction has been observed, a lotion of boric acid should be prescribed, and used until the inflammation has subsided.

A large number of observations have now been made on the use of this reaction clinically. Fortescue-Brickdale<sup>1</sup> collected the statistics relating to over 4,000 cases, with the following results: Of 1,623 cases clinically tuberculous, 1,419, or 87.4 per cent., gave a positive reaction; of 1,931 clinically non-tuberculous, 214, or 11.1 per cent., were positive; and of 710 which were doubtful, 275, or 38.7 per cent., gave the reaction. From this it would seem that the test is of considerable value as a means of recognizing the existence of tuberculosis, but is not infallible. Our personal experience bears out this view, and, moreover, suggests that the ophthalmic-reaction is more reliable than other tuberculin tests in the adult, as it less frequently gives a positive result in cases where the tuberculous lesion has satisfactorily healed.

On the other hand, it must be admitted that the procedure is not entirely free from risk. In an exceedingly

<sup>1</sup> *Bristol Med.-Chir. Journ.*, 1908, xxvi. 112.

small number of cases a severe conjunctivitis may result, and may last for fourteen days or more, causing the patient considerable inconvenience. In rare instances mechanical injury to the conjunctiva has appeared to be the starting-point of phlyctenular ulceration,<sup>1</sup> and in one case an incised wound of the cornea caused by the point of the pipette during instillation of the tuberculin was followed by sloughing of the cornea, hypopion, and destruction of the globe. Then, too, the solution, when exposed to the air, is an excellent cultivation-medium for bacteria, and the introduction of septic material into the conjunctival sac may give rise to troublesome symptoms. Care must therefore be taken to use sterile solutions. Any existing disease of the eye must be held an absolute bar to the use of the test, as it is impossible to foresee the extent of the inflammation that will ensue. Wolff-Eisner,<sup>2</sup> however, does not regard conjunctivitis as a contraindication; and Stephenson<sup>3</sup> even recommends the use of the test for cases of suspected tubercular disease of the eye.

On the whole, we are inclined to consider Calmette's test as of great value, as it is free from the risk of causing severe constitutional disturbance and consequent ill effects on the tubercular process elsewhere, and the danger of causing injury to the eye itself is exceedingly small when the test is properly applied.

<sup>1</sup> See Butler, *Brit. Med. Journ.*, 1908, ii. 304.

<sup>2</sup> "The Ophthalmic and Cutaneous Diagnosis of Tuberculosis." Tr. by Robert, 1908.

<sup>3</sup> *Brit. Med. Journ.*, 1907, ii. 1038.

## CHAPTER XVI

### TUBERCULOSIS (Concluded)

#### TREATMENT

**Nature of immunity in tuberculosis.**—It must be confessed that, in spite of the wide distribution of the disease, we have little definite knowledge as to the mode of resistance on the part of the host. That antibodies are formed is certain, as is shown by the phenomena of phagocytosis and opsonin-formation, of precipitation, of agglutination, and of complement-fixation, but we are ignorant of the part played by such bodies in overcoming infection. No satisfactory antitoxic or bactericidal serum has been prepared. For purposes of specific treatment three forms of the disease may be distinguished :

1. Acute generalized tuberculosis.
2. Tuberculosis of the lungs.
3. Localized tuberculosis of other organs, collectively known as "surgical tuberculosis."

1. Acute generalized tuberculosis is an acute toxæmia, with symptoms closely resembling those of enteric fever; it indicates an almost complete lack of resistance to the bacilli, and is met with in children and young persons. Theoretically, an antitoxic serum would be the only suitable remedy.

2. Tuberculosis of the lungs is typically a chronic localized infection, affecting organs which are richly supplied with blood and which cannot be kept at rest. Consequently, except in the most chronic cases, in which the disease is shut off by dense fibrous tissue, there is likely to take place

a constant escape of tuberculous poisons into the blood-stream (so-called "auto-intoxication"). Such a condition is not likely to be benefited by vaccine (tuberculin) treatment, and actually there is little evidence in favour of this procedure. The only type of case likely to receive benefit from such treatment is the localized focus with surrounding fibrosis—the apyrexial chronic consumptive.

3. Surgical tuberculosis is analogous to other localized infections, such as furunculosis or urethritis, and might theoretically be expected to prove suitable for vaccine treatment, little poison spontaneously reaching the general circulation, and little formation of antibodies being consequently excited. Inoculation treatment is therefore theoretically justifiable in this form of the disease, and in practice it is found that treatment with tuberculin is often strikingly successful.

**Therapeutic use of tuberculin.**—It is necessary to bear in mind that tuberculin of any kind is of the nature of a vaccine and not of an antitoxin ; it does not neutralize the poisons at work, nor does it directly kill the bacilli, but acts by stimulating the body to the formation of antagonistic substances. Therefore the principles already laid down as to the employment of vaccines apply to that of tuberculin. If, on the one hand, large doses of tubercular toxins are entering the patient's circulation, it is unreasonable and can only do harm to add more by giving tuberculin in any form. Now, the entrance of these toxins into the system is to be inferred—since they cannot be directly identified—from the clinical symptoms, such as pyrexia, wasting, loss of strength, and so forth. It is therefore theoretically inadvisable to use such a remedy in progressive, febrile cases of tuberculosis, wherever the disease is situated. Moreover, the production of antibodies in response to the tuberculin depends on the general nutrition of the patient, since antibody-formation is to some extent analogous to secretion and depends on the capacity of the tissue-cells to assimilate food-materials and build up the

required products. Therefore it is theoretically unlikely that an emaciated and exhausted patient will do well on tuberculin, as his power of reaction will be exhausted ; while it is necessary to carry out the accepted measures of hygiene—plentiful feeding and open-air life—during a course of tuberculin in order to increase the patient's reactive capacity.

There can be little doubt that the disappointing, often disastrous, results so frequently produced by the use of tuberculin, when it was first introduced to the medical world, were due to ignorance of the nature of the remedy and of the effects which could be rightly expected of it. Tuberculin has not—and was never supposed by its inventor to have—any power of replacing tissue already destroyed by the disease ; nor can it do anything to check the action of other bacteria, such as streptococci or staphylococci, which may have secondarily invaded the cavities in the lungs or other ulcerated lesions. We thus see that, just as in serum treatment it is important to administer the dose of antitoxin before the poisons of the bacteria have gained too long a start and entered into combination with the cells, so it is equally necessary, if permanent cure is to be effected, to make use of tuberculin (if at all) in the early stages of tubercular disease, before the substance of the affected organ has been so extensively destroyed as permanently to cripple the infected individual by the loss of an important structure. It is therefore in incipient tuberculosis that we must look for the most marked results from the administration of tuberculin. In more advanced cases of localized disease it may, indeed, be of assistance in increasing the resistance of the body to the tubercle bacilli, but permanent lesions will necessarily remain, and no cure in the truest sense can be hoped for.

A further reason for the original failure of tuberculin to come up to the expectations formed of it was that reliance was placed upon it, alone and unaided, to accomplish the cure of tubercular disease. Its value is now

recognized as an adjuvant to other remedial measures, not as a specific curative agent, such as is mercury for syphilis, or quinine for malaria. Used rationally in the light of modern experience, tuberculin is now proving itself a valuable remedy in certain forms of tuberculous infection.

**By-effects of tuberculin.**—The injection of the original tuberculin (T.) may be followed by the appearance of a rigor in some instances, and albumin may be found in the urine. Pains in the joints may occur, as after injection of serum. In some cases jaundice has resulted, and affections of the skin may be produced. Thus, purpuric eruptions have been recorded, and Thin<sup>1</sup> quotes an instance in which a generalized scarlatinal rash appeared, followed by desquamation.

The new tuberculin (T.R.) may also produce rigors, and severe headache may occur after an injection. Albuminuria is also met with, and may be considerable in amount.<sup>2</sup>

Cranston Low<sup>3</sup> records the appearance of a rash resembling lichen scrofulosorum, a phenomenon never yet observed by the authors. Weischer<sup>4</sup> saw an acute pleurisy arise apparently as a consequence of an injection of tuberculin, and we have more than once seen a similar occurrence as the result of too large a dose.

In a few instances death has followed an injection, and has been attributed to the action of the tuberculin.<sup>5</sup>

#### TUBERCULIN IN PULMONARY TUBERCULOSIS

The question of the value of tuberculin in the treatment of pulmonary tuberculosis is one of the greatest importance at the present time. Owing, to some extent, to recent legislation, which has brought the care and control of consumptive persons into the hands of the State, much attention has

<sup>1</sup> *Brit. Med. Journ.*, 1890, ii. 1330.

<sup>2</sup> Adrian, *Arch. f. Derm. u. Syph.*, 1898, Bd. xlv., p. 97.

<sup>3</sup> *Scott. Med. and Surg. Journ.*, Sept., 1905.

<sup>4</sup> *Zeitschr. f. Tuberk.*, 1905, Bd. vii., Hft. 3.

<sup>5</sup> See Adler, *Prag. med. Woch.*, 1904, No. 30, p. 389.

been concentrated on the problems presented by this disease while the medical supervision of these patients has been entrusted to special officers, many of whom have not previously had any prolonged experience of the affection with which they are called upon to deal. As a result, certain enthusiasts for the use of tuberculin have been able to secure for their views a large and uncritical audience, and the belief has been fostered that in tuberculin we have a remedy which is applicable to a large percentage of all cases of consumption, and which can be relied upon actually to cure this disease. We fear that very grave injury has resulted from this belief. Owing, perhaps, to the impossibility of keeping the lungs at rest, and to the free blood-supply of these organs—conditions which favour the free passage of tubercular poisons into the patient's circulation—pulmonary tuberculosis seems to stand, with respect to treatment, on a different footing from the localized forms of the infection met with in bones, joints, and glands, and in the genito-urinary tract. Whatever the cause may be, the number of cases of pulmonary disease in which good results appear to follow the use of tuberculin is so small that it is difficult to be sure that the amount of good done is sufficient to counterbalance the dangers that are inherent in the method. For it is beyond dispute that very great harm may be done by the ill-judged use of tuberculin in consumptive patients. An attack of pleurisy and an acute exacerbation of the disease may be set up by too large a dose, and the progress of the disease may be accelerated by injudicious injections. Indeed it may be said with some degree of probability that the discovery of tuberculin has resulted in evil rather than good for the sufferers from this form of tuberculosis. In any event, the number of cases suitable for tuberculin treatment is very strictly limited. If tuberculin is to be used at all in the treatment of consumptives—and it would be far preferable to discard it altogether than to admit anything approaching to indiscriminate use of so dangerous a substance—it



should be reserved for those who (1) are free from fever; 2) are in good general condition, so as to be capable of reacting satisfactorily in the direction of forming antibodies; and (3) are not making progress under those conditions of fresh air, plentiful diet, and regulated life which constitute the hygienic treatment of the disease.

If in such a case it be determined to try tuberculin, the initial dose must be exceedingly small, such as 0.00001 mg. T.R. or 0.000005 mg. B.E., the patient being kept in bed and watch maintained for any sign of reaction. During the course of injections attention must be paid to the general condition of the patient—weight, appetite, cough, etc.—as well as to the temperature chart. Little emphasis can be laid on his subjective sensations following the injections. Whether it be that tuberculin acts as a temporary stimulant, or that faith in an occult remedy has a similar effect, consumptive persons often assert that they are deriving marked benefit from these injections even when they are obviously declining in health. As an instance of the influence of faith in this connection may be quoted the case of a patient who persisted in attributing great benefit to injections of tuberculin while steadily deteriorating under this treatment. On the substitution of saline solution, without his knowledge, for the tuberculin, he still stated that he felt better after each administration and begged for a continuance of the treatment.

In the treatment of tubercular disease of the lung, the original tuberculin has obvious drawbacks. It causes reaction of the tissues round the lesions, and consequent casting-off of diseased material. In a deeply situated organ such as the lung, the cast-off matter cannot be readily expelled, and danger may ensue. Hence in pulmonary disease it would seem preferable to employ the *new tuberculin*; but both varieties have been used, and writers have not always distinguished between them. They must therefore be considered together.

In the following paragraphs we summarize some of the

more noteworthy views which have been published on this subject.

In a communication to the South California Medical Society, in 1903, Pottenger<sup>1</sup> gave the result of his own experience at that time, along with much information gained in answer to questions addressed to some of the principal authorities on the treatment of tuberculosis as to their experiences with this remedy. He found that of those who had actually used tuberculin, 60 per cent. were in favour of it as a means of treatment. Those who recommended the procedure based their advice on as many as 5,742 cases treated, whereas those who were of the opposite view had only a material of 813 cases to rely upon; indeed, only four of those who denied the value of the drug had at all an extensive experience of its use. This is, of course, only what might be expected, as those who found that they were getting no good results would cease using the tuberculin, while those who found it of value would persevere. It is noteworthy that of the four who had tried it extensively, and yet reported unfavourably on the whole, not one was actually opposed to its use; and all had apparently seen some cases at least in which good had been done.

In the majority of instances, those who had abandoned tuberculin treatment had not at any time given it an extended trial; whereas Petrushky maintained at the Berlin Congress that, in order to produce lasting effects, the treatment should extend over several years, a course of a few months being taken each year.

Coming to actual results claimed for treatment with tuberculin in addition to ordinary measures, we find that, in addition to his own success, Pottenger quotes five other physicians who claim to have cured 100 per cent. of those cases which came under treatment with tuberculin in the earliest stage of the disease (Jessen, Turban, Wilkinson,

<sup>1</sup> *Therapeutic Gazette*, 1903, p. 163. The references to other authors in the following paragraphs are taken from this article.

Klebs, Petrushky). Von Ruck claims 93 per cent., Trudeau 83 per cent., and Rembold 75 per cent. of cures in similar cases. In all, 589 cases treated with tuberculin came under consideration, with a proportion of cures equivalent to 84·2 per cent. On the other hand, among 611 collected cases which were treated in the ordinary way without the aid of "culture products," 391, or 64 per cent., were regarded as cured.

The results obtained by individuals with and without tuberculin, as quoted by the same writer, are of considerable interest. Trudeau, in his first report on the remedy, recorded 24 cases treated with tuberculin, with a percentage of cures of 83. Among 113 cases treated without it he cured 72 per cent., giving a difference of 11 per cent. in favour of the remedy. More recently he gives the results of 94 cases, 47 treated with, and the same number without, tuberculin; of the former group 41 were cured, of the latter 36—again a small difference in favour of Koch's preparation.

Turban gives details of his results in cases which came under treatment in the first, second, and third stage<sup>1</sup> of the disease respectively. Taking the last first: he found that, whereas the mortality in cases treated without tuberculin

<sup>1</sup> For purposes of classification in statistics of sanatoria, etc., pulmonary tuberculosis is divided into three stages. Different authorities have devised slightly different methods of classification. That of Turban, which may be taken as typical, is into—*First stage*: Cases in which only one lobe is affected, or only portions of two lobes equivalent to one lobe in extent. *Second stage*: Cases in which two lobes are extensively involved. *Third stage*: Cases in which the disease is still further advanced. It will be seen that this classification is purely arbitrary, and merely affords a rough indication of the severity of individual cases. It does not correspond at all with the well-known pathological division into the stages of (1) tubercular deposit, (2) consolidation, (3) excavation or cavity-formation. It is practically impossible to ascertain with any exactitude the extent of the pathological changes in the lung from a study of the physical signs; the pathological classification is therefore not available for practical use.

was 50 per cent. within a period of two years, among those treated with tuberculin only 25 per cent. died within the same time-limit. He did not find that tuberculin had any tendency to induce attacks of hæmorrhage in these cases, nor did it ever give rise to a generalized tuberculosis. Tubercle bacilli disappeared from the sputum in four cases out of 21 in which tuberculin was used. Of course a real cure was not to be hoped for in patients coming for treatment at so advanced a stage of the disease.

Of 48 patients in the second stage of the disease, treated with tuberculin, 36 were alive four years afterwards; whereas, of 152 who did not receive injections, 107 survived for the same length of time. The figures do not themselves prove much in favour of tuberculin, but Turban considers that the actual condition of the various patients afforded strong evidence of its value. Of cases which came under treatment in the first stage of the malady, Turban, as already stated, claims 100 per cent. of cures. In all the cases in this stage of the malady in which tubercle bacilli were at first found in the sputum, they disappeared under treatment. Taking this last as a test of the value of tuberculin, he shows that of a total of 86 cases so treated, 45 (52 per cent.) were permanently freed from the organisms; whereas, of 241 patients not so treated, 95 (39 per cent.) only were similarly benefited.

Denys made trial of tuberculin alone, without the aid of other remedial measures, such as rest, open air, and medicines. He claims to have cured in this way 29 per cent. of his cases (174), and greatly benefited another 42 per cent. As was previously pointed out, there is no reason, except for purely experimental purposes, to suspend ordinary hygienic measures during the administration of tuberculin; in order to produce the maximum of advantage to the patient the two should be combined.

Wurtzen<sup>1</sup> recorded good results obtained in 10 cases with the *old tuberculin*, given according to the rules

<sup>1</sup> *Tuberculosis Bull. Mens.*, Feb., 1904, p. 53.

advised by Goetsch,<sup>1</sup> viz. never to inject febrile patients; never to increase the dose till the previous amount can be tolerated without reaction; and to insist on rest in bed on the day of treatment and the following day.

Within the last decade, largely owing to the work of Wright, a considerable impetus has been given to the use of tuberculin in pulmonary tuberculosis, and with the adoption of smaller doses the proportion of successful results would seem to have risen. The majority of writers on the subject are in favour of the employment of the remedy.<sup>2</sup> Nevertheless it is very difficult to obtain valid evidence of actual benefit arising from its use in this form of tubercular disease, and voices of might have recently been raised on the other side. Karl Pearson, as the result of an extended study of statistical material available at King Edward's Sanatorium at Midhurst, concludes that no proof of beneficial action is therein afforded. Bardswell<sup>3</sup> concludes "that the administration of tuberculin is quite unsuitable as a routine method of treatment for all cases of pulmonary tuberculosis, and that its indiscriminate and careless use on a large scale can only end in disaster."

There is also a difference of opinion as to what cases are best adapted for the treatment. Thus, Amrein<sup>4</sup> and Roemisch<sup>5</sup> would restrict the use of the remedy to chronic cases without fever, and Lawson and Stewart<sup>6</sup> agree with this advice; whereas Krause<sup>7</sup> and Hammer<sup>8</sup> do not regard fever as a contraindication. Hæmoptysis is not always

<sup>1</sup> *Deut. med. Woch.*, June 20, 1901.

<sup>2</sup> For further information on the use of tuberculin, reference should be made to the treatises—by Sahli, "Ueber Tuberkulinbehandlung"; by Bandelier and Roepke, "Tuberkulinbehandlung und Tuberkulose-immunität"; and by Riviére and Morland, "Tuberculin Treatment."

<sup>3</sup> *Lancet*, 1915, i. 68.

<sup>4</sup> *Beitr. z. Klin. d. Tuberk.*, Bd. iv., Heft 2.

<sup>5</sup> *Münch. med. Woch.*, 1906, No. 3.

<sup>6</sup> *Lancet*, 1905, ii, 1679.

<sup>7</sup> *Münch. med. Woch.*, 1905, No. 32.

<sup>8</sup> *Ibid.*, 1906, p. 2423.

looked upon as a bar to the cautious use of the new tuberculin for remedial purposes, though it is so to the diagnostic use of the old tuberculin: it is even suggested that tuberculin is valuable as an agent for arresting hæmorrhage. A few writers still recommend the old tuberculin for purposes of treatment (Foss,<sup>1</sup> Jacquerod<sup>2</sup>); but "T.R." is the form usually adopted. The emulsion of the bacilli is preferred by some (Krause,<sup>3</sup> Elsaesser,<sup>4</sup> Poppelmann<sup>5</sup>), and has also been used by Wright. It has the advantage of cheapness.

Great caution must be observed and very minute initial doses given, if it be decided to administer the remedy to patients who suffer from pyrexia, as considerable harm may be done by doses which excite any strong reaction. The question of dosage is discussed later (pp. 334-6).

#### TUBERCULIN IN SURGICAL TUBERCULOSIS

We have already mentioned that it was as a cure for consumption that tuberculin was first announced to the world, and that, when the extravagant hopes thus raised were disappointed, the pendulum swung too far in the opposite direction, and the valuable properties of the preparation were overlooked. Some attempts were indeed made to maintain the value of tuberculin in the treatment of lupus; but as a remedy in other kinds of tuberculosis it fell into entire disuse. Yet there is little doubt that in some at least of the varieties of lupus very favourable results may be obtained by a proper use of tuberculin, or that it deserves trial in all obstinate cases which resist other remedies. As we have already pointed out, the respective actions of the old tuberculin and of the new (T.R.) are quite distinct; hence they must be considered separately.

<sup>1</sup> *Zeitschr. f. Tuberk.*, Bd. vi., Heft 5.

<sup>2</sup> *Rev. Méd. de la Suisse Romande*, 1906, No. 2.

<sup>3</sup> *Op. cit.*

<sup>4</sup> *Deut. med. Woch.*, 1905, No. 48.

<sup>5</sup> *Berl. klin. Woch.*, 905, No. 36.

**Tuberculin in the treatment of lupus vulgaris.**—

Very good results were claimed in this disease from the use of the *old tuberculin* when it was first introduced (1890). Koch<sup>1</sup> in his original paper wrote as follows: "A few hours after the injection into the skin of the back . . . the lupus-spots begin to swell and redden; and this they generally do before the initial rigor. During the fever swelling and redness increase, and may finally reach a high degree, so that the lupus-tissue becomes brownish and necrotic in places. Where the lupus had been sharply defined we sometimes found a much-swollen and brownish spot surrounded by a whitish edge about a centimetre wide, which again was surrounded by a broad band of bright red. After the subsidence of the fever the swelling of the lupus-tissue decreases gradually, and disappears in about two or three days. The lupus-spots themselves are then covered by a crust of serum, which filters outwards and dries in the air; they change to crusts, which fall off after two or three weeks, and which sometimes leave a clean cicatrix behind after one injection. Generally, however, several injections are required for the complete removal of the lupus-tissue. . . . There is no question of the destruction of the tubercle bacilli in the tissues; it is only the tissue enclosing the tubercle bacilli which is affected by the remedy."

Striking results were also recorded by other observers. Thus, Saundby, Simon, and Gilbert,<sup>2</sup> in a communication to the *Birmingham Medical Review*, though speaking cautiously of the results achieved by the use of the remedy, yet allude to remarkable improvement as taking place in this disease; and Heron, at the Medical Society of London, stated that this was so marked that tuberculin would soon be regarded as an essential in the treatment of lupus. Barling<sup>3</sup> recorded 14 cases of lupus treated by this means,

<sup>1</sup> *Deut. med. Woch.*, 1890; *Brit. Med. Journ.*, 1890, ii. 1193.

<sup>2</sup> *Brit. Med. Journ.* Epitome, Dec. 20, 1890, p. 92.

<sup>3</sup> Quoted in *Brit. Med. Journ.* leading article, April 25, 1891, p. 922.



of which 4 were very much improved, 8 considerably so, 2 slightly benefited. Soon, however, less favourable reports began to come to hand. It was found that, though the first effects were encouraging, relapses were very liable to occur. Radcliffe Crocker pronounced the remedy disappointing on the whole, and this verdict was generally accepted. Consequently tuberculin fell into disuse among the body of the profession as a method of treating lupus.

A few cases are, however, still recorded from time to time in which good results are obtained from the use of the old tuberculin. As an example we may quote the salient facts of a case reported by E. F. Maynard,<sup>1</sup> which illustrates the use of the remedy. The patient was a cook, aged 40, who had suffered from lupus of the nose for some time, and had been treated for the past three years by scraping and cautery (acid nitrate of mercury, fuming nitric acid, etc.) without permanent benefit. The disease was advancing, and had involved the septum nasi. "Old tuberculin" was administered, beginning with doses of 0.001 c.c. injected into the arm. The site of injection became red, swollen and painful, and the temperature rose from 99.8° F. to 102.4° F. There ensued headache, nausea, and feeling of illness, and the nose became painful, red, and swollen. The doses were increased gradually. After 0.005 c.c. had been reached there was no further reaction till 0.007 c.c. was given. Then again no reaction occurred till a dose of 0.03 c.c. was reached. After 0.09 c.c. had been administered no further reaction was seen, though 0.1 c.c. was given several times over. The disease healed up entirely, and no relapse had occurred seventeen months afterwards.

In the above case the treatment by tuberculin alone, without the adoption of any other measure, seems to have effected a cure. The majority, however, of those who are in favour of the use of tuberculin recommend that it should be employed along with surgical measures, such as scraping.

<sup>1</sup> *Brit. Med. Journ.*, 1900, ii. 1777.

In some instances it has been combined with the administration of thyroid extract, apparently with good results.

*Local use.*—A method of applying tuberculin locally has been devised by Verge,<sup>1</sup> who makes a 5-per-cent. ointment of old tuberculin with soft paraffin, and rubs it into the lesions for one to two minutes, after preliminary cleansing with a starch poultice. Lint smeared with the ointment is then applied under a bandage for a period of twenty-four hours. The application is repeated daily for three or four days. There may be considerable pain, and a peculiar odour is evolved. Healing takes place in about ten days.

On the introduction of the *new tuberculin* (T.R.) it was tried in lupus by a number of observers. Thus, Bussenius<sup>2</sup> reported 3 out of 4 cases improved, and Worner<sup>3</sup> 4 patients all benefited, especially 2 who suffered from lupus hypertrophicus. Doutrelepon<sup>4</sup> treated 15 cases with improvement in all, and van Horn<sup>5</sup> 10 with equally good results.

Adrian gives a detailed account of his treatment of 12 cases, of which 8 were apparently cured, and 4 did not entirely yield to the remedy. The table on the next page is taken from one of his articles, the results being added in a separate column.

The rise of temperature following the injections was generally marked; and sometimes a rigor occurred. The headache and general feeling of illness were parallel to the rise of temperature. The latter was more often met with after use of some particular specimens of tuberculin, and was more marked in some patients than in others. It was found that, if one dose had produced too great a reaction, it was necessary to reduce the amount used to a figure very much lower, even to a point below that at which no reaction

<sup>1</sup> *Brit. Med. Journ.*, 1910, ii. 2023.

<sup>2</sup> *Deut. med. Woch.*, 1897, No. 28, p. 441.

<sup>3</sup> *Ibid.*, No. 30, p. 476.

<sup>4</sup> *Ibid.*, No. 37, p. 537.

<sup>5</sup> *Ibid.*, No. 39, p. 625.

Case	Sex and age		Dose		No. of injections	Duration of treatment	Result
1	Female	19	1/1000	increased to 20	50	132 days	Cured.
2	"	20	1/500	" 20	43	129 "	"
3	"	17	1/500	" 20	35	115 "	"
4	"	39	1/1000	" 20	47	143 "	"
5	"	27	1/500	" 20	36	114 "	"
6	Male	32	1/500	" 20	36	87 "	"
7	Female	14	1/1000	" 20 ( $\times 4$ )	55	193 "	"
8	"	6	1/1000	" 20	61	216 "	"
9	"	48	1/500	" 12	67	199 "	Not cured
10	"	14	1/500	" 2.5	68	156 "	"
11	"	35	1/500	" 2/10	31	84 "	"
12	"	56	1/1000	" 4/10	49	155 "	"

had previously been met with. Transitory febrile albuminuria was not uncommon, and in one case severe albuminuria occurred. Hyaline casts were sometimes found in the urine. In no instance did a local abscess ensue at the site of injection, nor was there urticaria, herpes, or enlargement of glands. No local reaction is seen at the site of the lupus-lesions when the new tuberculin is employed—a contrast with the old tuberculin.

In spite of these apparently good results, Adrian is not very favourable to the use of tuberculin (T.R.). It is exceedingly expensive, which is undoubtedly a drawback to its use. Adrian used altogether 188 c.c. of the fluid in a total of 578 injections, the cost being 1,598 marks, or, approximately, £80. He recommends the employment of surgical measures as well as the tuberculin, and does not consider the new preparation any better than the old.

Mayer<sup>1</sup> practically agrees with this verdict, holding that tuberculin may do good in lupus, but that it is not superior to ordinary measures; while Bussenius and Cossmann<sup>2</sup> report that no constant improvement occurs in

<sup>1</sup> *Arch. f. Derm. u. Syph.*, 1898, xlii. 267.

<sup>2</sup> "Das Tuberculin T.R. und sein Wirkung." Berlin, 1898. (*Cf.* Bussenius, *Deut. med. Woch.*, 1897, No. 28, p. 441.)

all cases. "It cannot be denied," they write, "that Koch's T.R., injected in accordance with Koch's directions, may have a good effect on a focus of lupus; yet our failures and the negative results recorded by others show that such a favourable result is not an absolute certainty." On the other hand, Brocchieri<sup>1</sup> considers that the new tuberculin is superior to the old in the treatment of lupus, and may succeed where the latter has failed. He thinks that the spread of the disease is prevented by its employment. The duration of treatment should not be less than one year. More recently favourable reports of its value have been given by Bulloch<sup>2</sup> and by Darier.<sup>3</sup> Bandelier<sup>4</sup> found benefit to result from the use of "persucht tuberculin."

On theoretical grounds it would seem reasonable to make use of the two forms of tuberculin in conjunction for the treatment of lupus—using the old preparation until the necrotic tissues are thrown off, and then administering the new tuberculin in order to produce immunity, and thus prevent subsequent relapse and spread of the disease.

**Tuberculin in tubercular laryngitis.**—Very much the same results were obtained by the use of the *old tuberculin* in laryngeal phthisis as in the case of lupus. Good effects were at first reported, as by Struebing,<sup>5</sup> who recorded a case in which great benefit ensued as the result of this treatment. There was at first a period in which there were increased hoarseness, and pain in the throat—effects of the local reaction. After nine injections the surface of the lesions looked cleaner and healthier, final cicatrization being produced after forty-three doses of the remedy. The ulceration ultimately seemed to be entirely cured. Lennox Brown<sup>6</sup> reported Gerhardt's results in 19 cases, 17 of which

<sup>1</sup> *Il Policlinico*, 1898, No. 21, p. 489.

<sup>2</sup> *Lancet*, 1905, ii. 1603.

<sup>3</sup> *Ann. de Dermatol.*, 1905, p. 249.

<sup>4</sup> *Beitr. z. Klin. der Tuberk.*, vi. 115.

<sup>5</sup> *Deut. med. Woch.*, Oct. 8, 1891.

<sup>6</sup> *Brit. Med. Journ.*, 1890, ii. 1485.

were much improved, only 2 failing to receive any benefit. Senator<sup>1</sup> also reported marked improvement in cases which he had treated. The same doubt, however, as to the permanence of the good effects produced by tuberculin exists in this disease as in lupus, and it is not now often employed.

Soon after the *new tuberculin* was introduced, Hersfeld<sup>2</sup> recorded 7 cases in which he made use of it. He noted that the solutions keep badly, and that a glycerin solution is more painful to the patient than a saline solution. Bandelier and Roepke<sup>3</sup> find the remedy of great value in this affection, practically doing away with the need for local treatment in early cases. Pottenger<sup>4</sup> found von Ruck's tuberculin of use, and Krause<sup>5</sup> used the emulsion of bacilli with advantage.

We have personally treated 6 cases of tuberculous laryngitis with T.R., and in 4 of these, where the disease was localized to the larynx, complete recovery, with strong, though harsh, voice, took place; but in 2 cases associated with extensive lung-mischief, although laryngeal improvement followed the use of the remedy, the pulmonary lesions progressed and death ensued within twelve months.

**Tuberculin in disease of bones and joints.**—The *old tuberculin* produces phenomena of swelling and redness around tuberculous joints, just as it does around patches of lupus. Koch<sup>6</sup> reported as follows in his original paper:—"Glandular, bone-, and joint-tuberculosis were similarly treated, large doses at intervals being employed. The result was the same as in the lupus-cases—a speedy cure in recent and slight cases, and slow improvement in severe cases." In spite of encouraging results recorded by some authors at first, the general verdict was ultimately

<sup>1</sup> *Berl. klin. Woch.*, Dec. 10, 1890.

<sup>2</sup> *Deut. med. Woch.*, Aug. 19, 1897, p. 543.

<sup>3</sup> *Op. supra cit.*

<sup>4</sup> *Amer. Journ. Med. Sci.*, Dec., 1906.

<sup>5</sup> *Münch. med. Woch.*, 1905, No. 32.

<sup>6</sup> *Loc. cit.*

unfavourable to the use of tuberculin in these cases, most observers apparently agreeing with Edmund Owen that the final results gained were no better than could be produced by rest alone.

Of the *new tuberculin*, Adrian<sup>1</sup> reported that it had no effect on disease of bone or glands. On general grounds it seems unlikely that, if it raises the general resisting-power of the body, it should have no effect on these special forms of the disease. No doubt it is difficult to ascertain the exact amount of improvement produced by it, as it has no local effect of a visible kind. Theoretically it would seem that the new rather than the old tuberculin should be tried in these cases, as they are deeply seated, and there is no means of escape for the necrotic material, if it be cast off as a result of treatment with old tuberculin.

Recently good results have been reported by Gray<sup>2</sup> and by Low.<sup>3</sup> A case of hip-disease benefited by tuberculin is recorded by Crofton,<sup>4</sup> and our own observation of a considerable number of cases of tuberculous hips, knees, and wrists, treated either in the wards of a hospital or as out-patients, leads us to regard T.R. as a most valuable remedial agent resulting in the majority of cases in complete cure, the affected joints being freely movable.

**Tuberculin in ophthalmic disease.**—Eyre and Ormond<sup>5</sup> record complete cure following the use of T.R. in a case of extensive tuberculous disease of the conjunctiva. Eyre<sup>6</sup> has subsequently reported a series of 11 cases, of which 8 were cured, 2 much improved, and 1 not benefited. Reuchlein<sup>7</sup> reports favourably on the use of the preparation in ocular diseases (iritis, keratitis, disease

<sup>1</sup> *Op. cit.*

<sup>2</sup> *Lancet*, 1906, i. 1099.

<sup>3</sup> *Brit. Med. Journ.*, 1908, i. 550.

<sup>4</sup> *Lancet*, 1908, ii. 731.

<sup>5</sup> *Trans. Ophthalm. Soc.*, 1907, p. 27.

<sup>6</sup> Hunterian Lectures, R.C.S., 1912; *Lancet*, 1912, i. 1319.

<sup>7</sup> *Klin. Monatsh. f. Augenheilk.*, 1906, i. 352.

of the ciliary body). Erdmann<sup>1</sup> also used this method of treatment with advantage, and we have seen considerable benefit accrue in cases of tubercular iritis, of tubercles in the choroid, and of tuberculous periostitis of the orbit.

**Tuberculin in genito-urinary and peritoneal disease.**—In the experience of the present writers, tuberculin (T.R.) finds its most successful application in tuberculous infections of the genito-urinary tract—indeed, where the disease is bilateral and involves the bladder as well, so that surgical measures are out of the question, the administration of tuberculin holds out the only hope of prolongation of life. One of our patients, whose life could to all appearances be measured by weeks only, recovered sufficiently to lead a useful life for another two and a half years.

Pardoe<sup>2</sup> recommends the use of tuberculin in cases of tuberculosis of the bladder, ureters, and kidneys. Birnbaum<sup>3</sup> also reports good results in cases of tuberculosis of the bladder, kidney, and uterine adnexa, and in tubercular peritonitis. Bandelier and Roepke<sup>4</sup> confirm the value of tuberculin in the peritoneal affection.

For dosage *see* pp. 334-6.

## VACCINATION AGAINST TUBERCULOSIS

**Attenuation of tubercle bacilli.**—For the purpose of vaccination against any disease, the first requisite is the preparation of an attenuated form of the causal organism, and for a long time it seemed as if it were impossible to reduce the virulence of the tubercle bacillus. Many observers<sup>5</sup> have, however, now succeeded in the endeavour to attenuate this organism, and in the case of the lower animals it has been claimed that immunity can be produced

<sup>1</sup> *Münch. med. Woch.*, 1907, p. 671.

<sup>2</sup> *Lancet*, 1905, ii. 1766.

<sup>3</sup> *Centralbl. f. Gynäk.*, 1907, No. 3.

<sup>4</sup> *Op. supra cit.*

<sup>5</sup> Salmon (*Philadelphia Med. Journ.*, June 13, 1903, p. 966) gives an historical summary of the results obtained in attenuating the tubercle bacillus. The following account is principally taken from his paper.



by means of such cultures. In 1889 Darenburg<sup>1</sup> inoculated rabbits with dead cultures of tubercle bacilli, and found that, though they were at the time made ill by the injections, yet afterwards they were more resistant to infection with virulent bacilli. In the same year Grancher and Martin<sup>2</sup> prepared a series of cultures of different degrees of virulence, and stated that they had succeeded in immunizing rabbits by this means against the disease.

In 1890 Trudeau<sup>3</sup> gave an account of two cultures of tubercle bacilli of very different virulence. The first was from the lung of a man who had died of miliary tuberculosis. It grew very slowly on glycerin-agar in isolated scaly masses. The second was from a guineapig which had been inoculated with bacilli from an old phthisical cavity, and the bacilli had been grown for a long time on artificial media. This culture grew rapidly, forming a thick, creamy pellicle on the surface of the medium. It was much less virulent for rabbits than the former. Trudeau failed, however, to produce immunity by injection either of culture-products or of attenuated organisms. In the year 1894 he announced that rabbits inoculated with avian bacilli seemed to gain a certain amount of additional resistance to the human form. Guineapigs, which are scarcely susceptible to the avian bacillus, are not protected by injections of it against infection with other forms.

In 1894 de Schweinitz, by repeated subcultures, had produced a bacillus which was so attenuated that it no longer produced tuberculosis even in guineapigs, and by inoculating the animals with these attenuated bacilli, and afterwards with others of gradually ascending degrees of virulence, he immunized them against bovine bacilli. In 1897 the same observer showed that by injection with human tubercle-bacilli cows could be rendered immune to the bovine bacillus.

It is interesting to notice that the better a variety of the

<sup>1</sup> *Bull. de l'Acad. de Méd.*, Oct. 29, 1889, p. 391.

<sup>2</sup> *Ibid.*, Aug. 20, 1890.

<sup>3</sup> *Trans. Assoc. Amer. Physicians*, 1890, v. 183. *Ibid.*, 1894, ix, 168.

tubercle bacillus grows on artificial media, the less virulent it appears to be. The artificial pabulum constitutes a new environment to which the organism has to get accustomed, and as it does so it loses its original power of acting as a parasite. This forms a good example of the variation of bacteria according to their surroundings.

Behring<sup>1</sup> claims that by injection of human tubercle-bacilli into cattle he has produced immunity to the bovine form of the disease. The procedure, which he speaks of as "Jennerization," is harmless to the animals, and they subsequently resist not only artificial inoculation with their own form of tuberculosis, but also infection in the ordinary course of nature when they are brought into contact with other animals suffering from the disease. The duration of the immunity thus conferred is not yet certainly known; a second vaccination may be necessary subsequently. Behring suggests that this latter might be performed with modified bovine bacilli.

Friedmann<sup>2</sup> has made use of bacilli derived from the tortoise for immunizing warm-blooded animals; and Moeller<sup>3</sup> has experimented with similar bacilli from the slow-worm. Both authors record good results; but the method has not been tried on man.

In human beings prophylactic injection of attenuated bacilli does not seem to have as yet been attempted; while it is evident that the danger of using living tubercle-bacilli as a vaccine for human beings is too great to be faced. Maragliano,<sup>4</sup> however, announces that he has prepared a vaccine of a non-living nature, which he has employed on human beings, but the exact mode of preparing this material is not stated in his communication. The use of it

<sup>1</sup> *Zeitschr. f. Tiermedizin*, Bd. vi., Hft. 5 u. 6.

<sup>2</sup> *Therap. Monatsh.*, March, 1904, p. 123. Cf. Friedmann's tuberculin, p. 281.

<sup>3</sup> *Zeitschr. f. Tuberk. u. Heilst.*, Jan., 1904.

<sup>4</sup> Communication to the International Medical Congress, Madrid, 1903; *Med. News*, July 4, 1903, p. 1.

is said to result in an increase of the agglutinative power of the blood-serum, and also in a marked leucocytosis. These are the changes that have been observed in the blood of animals which have been immunized against the tubercle bacillus experimentally. The injections of Maragliano's vaccine are followed in human beings by the development of a small tubercular ulcer at the point of inoculation, accompanied by a form of suppuration which is bacteriologically sterile. There is fever for a few days, but no other ill effect. Behring<sup>1</sup> suggests that it may be possible to immunize young children prophylactically with antibodies derived from animals which have been injected with attenuated bacilli, and also administers tulase (p. 280) in milk for the same purpose.

Von Ruck used his tuberculin (p. 278) for immunization, and treated altogether 339 children, giving 0.05 c.c. to nurslings, and 0.2–0.6 c.c. to older children.

#### VACCINES IN TUBERCULOSIS

Gray<sup>2</sup> has used streptococcic and staphylococcic vaccines in cases of tuberculosis of bones, joints, and glands with advantage; the doses being 500–1,000 millions of staphylococci, and 100–250 millions of streptococci. We find this method of treatment extremely valuable in all forms of tubercular lesions which have become secondarily infected with pyogenic bacteria.

Habershon<sup>3</sup> speaks highly of the value of vaccines of *Micrococcus catarrhalis*, *Diplococcus pneumoniae*, and the pneumobacillus of Friedländer in cases of pulmonary tuberculosis with secondary infection, but finds the results disappointing in the presence of pneumococcus and streptococcus.

#### ADMINISTRATION OF TUBERCULIN

At the present time there are two chief methods of administering tuberculin. The first—that originally prac-

<sup>1</sup> *Berl. klin. Woch.*, March 16, 1903.

<sup>2</sup> *Lancet*, 1906, i. 1099.

<sup>3</sup> *Bristol Med.-Chir. Journ.*, June, 1914.

tised by Koch—consists in the rapid increase in the dosage at short intervals in the attempt to produce a tolerance of the remedy; and the second, which results from the teaching of Wright, consists in the administration of a series of small doses increased in size very gradually at considerable intervals, with the object of producing a real immunity. Each method has secured numerous advocates, and even now the difficulty of collating any really considerable number of cases with a view to obtaining statistical evidence of a reliable character is so great that the relative merits of the two methods remain an open question. Our own predilection is for that method which attempts to produce a real immunity rather than the one which merely aims at a toxin-tolerance.

#### CHOICE OF TUBERCULIN, AND DOSAGE

The various modifications of tuberculin may be classed under one of three forms :—

1. Those containing the soluble products of the tubercle bacillus or exotoxins, e.g. Old Tuberculin.

2. Those containing bacterial protoplasm or endotoxins only, e.g. New Tuberculin.

3. Those containing both exo- and endotoxins, e.g. Bacillary Emulsion.

In ordinary vaccine work the object aimed at is the production of antibodies capable of destroying the responsible bacteria and completely neutralizing all their toxic activities, whether endogenous or exogenous. Hence, on theoretical grounds preference should be given to the bacillary emulsion—the ideal preparation, of course, being the sensitized B.E., if only one could be satisfied that the sensitization was adequate and complete. Consisting as it does of solid particles, it is absorbed slowly and at an uncertain rate. But for practical purposes we may regard the bacillary emulsion as a combination of the old and new tuberculins (T. + T.R.), and personally we consider the dangers and disadvantages of old tuberculin when used as a

therapeutic agent completely to outweigh its possible utility. We have no hesitation in expressing our preference for the new tuberculin (T.R.), in spite of its cost, reserving the bacillary emulsion for the final stages of a satisfactory course of the former.

Koch advised, for the treatment of phthisis with the old tuberculin (T.), that the initial dose should be 0.001 c.c., and that this should be repeated about every second day till no temperature reaction followed its use. Then the dose should be raised to 0.002 c.c., and so on, rising to 0.01 c.c., etc. In strong individuals it might be possible to raise the doses more quickly. In lupus the first doses might be larger, 0.01 c.c., gradually raised. As we do not advise the use of this preparation therapeutically, we need not discuss the dosage.

The new tuberculin is supplied in liquid condition. It is an opalescent liquid similar in appearance to the mixture of five or six drops of milk with half an ounce of water. It must be kept in a cool, dark, and dry store; the solution contains the soluble protoplasm from 10 mg. of dried bacilli in each cubic centimetre—the actual amount of solid substance entering into solution being 2 mg.<sup>1</sup>

<sup>1</sup> For dilution of the liquid 20-per-cent. glycerin solution should be employed. This is prepared by boiling 20 c.c. pure glycerin with 80 c.c. distilled water for fifteen minutes, and then cooling thoroughly before use. The dilutions are preferably made in the following manner:

1. With a 1 c.c. pipette, calibrated to 1/10th, 0.3 c.c. is withdrawn from the bottle, and mixed with 2.7 c.c. 20-per-cent. glycerin solution, making a 10-per-cent. dilution.
2. From this 10-per-cent. dilution 0.1 c.c. is taken and made up to 10 c.c. with glycerin solution. Thus a 1-per-mille dilution of the original fluid is obtained. Two divisions or 2/10th c.c. of a Koch or Pravaz syringe of this dilution therefore contains the initial dose, 0.0002 c.c., of the original fluid.

Dilutions which present a turbid appearance, or show a deposit which does not dissolve upon shaking, must not be employed. Generally the dilutions keep well for a fortnight in a cool and dark place.

With the new tuberculin (T.R.) the initial dose, according to Koch, should be 0.002 mg. in fairly strong persons (equivalent to 0.0002 c.c. of the preparation, and containing actually 0.0004 mg. of solid substance); in very weakly subjects it is well to start with 0.001 mg. The dose is to be repeated every other day, increasing at such a rate as to avoid reaction as far as possible; the amount may usually be doubled each time.

Doutrelepon<sup>1</sup>, who tried this method, came to the conclusion that this rate of increase was too rapid. He advised that 0.002 mg. should be used for the first dose, 0.004 for the second, 0.006 for the third, and so on, up to 0.02 mg. Then the dose is to be increased by 0.02 each time up to 1 mg. The dose should not be repeated till the temperature has fallen to normal after the previous reaction, and the largest dose used by Doutrelepon was 4 mg. (0.4 c.c. of the preparation). The fresher the solution, the more likely is the occurrence of a marked reaction.

Rosenberger<sup>2</sup> gives 0.002 mg. of the new tuberculin to start with. The administration is followed by little febrile disturbance, but there may be some headache and sleeplessness. The appetite is increased by the treatment. The dose is gradually raised till 1 mg. is reached; then the old tuberculin is begun, the initial dose of the latter being 0.01 c.c.

Recent practice in this country has tended in the direction of giving much smaller doses of tuberculin than were formerly used. Thus, whereas 0.002 mg. of T.R. was considered a small amount to give as an initial dose, now 0.001 mg. would be looked upon as a large dose, and 0.00025, 0.0002, or 0.0001 mg. is more often given at first. Still more minute quantities are believed by some physicians to be capable of producing a definite reaction and to form effective doses for therapeutic use (e.g. 0.00005, 0.00002, or 0.00001 mg.). The object to be aimed at is to administer that amount which will just fail to produce

<sup>1</sup> *Deut. med. Woch.*, Aug. 19, 1897, p. 537.

<sup>2</sup> *Centralbl. f. inn. Med.*, 1903, No. 19, p. 465.

an appreciable reaction in the form of a rise of temperature, headache, sleeplessness, or other constitutional disturbance. The first doses therefore should always be small (e.g. 0·00005 mg.), and they should be raised very gradually until some signs of reaction occur. Thus, a course of injections might be given as follows: 0·00005, 0·0001, 0·0002, 0·0004, 0·0005, 0·001 mg. Or a small dose such as 0·0001 mg. may be maintained throughout. We have seen a harmful reaction ensue after a dose of 0·001 mg.<sup>1</sup>

Another change in the method of administering tuberculin which has been effected in recent years is the tendency to prolong the intervals between the separate doses. Thus, whereas they were originally given daily, or on alternate days, it is now usual to let an interval of 7, 10, 14, or even 21 days elapse between the injections. Sometimes it is preferred to give very small doses on two consecutive days, and then to wait for an interval. No definite rules can be laid down for all cases: each must be judged according to the special phenomena presented.

That tuberculin given *by the mouth* is absorbed and produces similar effects to those which follow hypodermic injection was affirmed by Freymuth;<sup>2</sup> but Köhler,<sup>3</sup> who tried this method of administration for therapeutic purposes, was not impressed by its advantages. Calmette and Breton<sup>4</sup> state that it is also absorbed by the bowel. More recently Latham<sup>5</sup> has used the oral method extensively, and upholds the value of this procedure. For such use the tuberculin (T.R.) is diluted with normal saline solution. It can be given in milk, or in any flavoured water if this is

<sup>1</sup> These doses represent the weight of dried tubercle bacilli used in their preparation. To ascertain the actual weight of soluble protoplasm contained in each, it is necessary to divide the figure given by 5—e.g. 0·0001 mg. = 0·00001 c.c. tuberculin, and contains 0·00002 mg. bacterial protoplasm.

<sup>2</sup> Quoted by Köhler.

<sup>3</sup> *Zeitschr. f. Tuberk.*, 1907, Hft. 4.

<sup>4</sup> *Compt. Rend. Acad. Sci.*, cxlii. 11. Cf. Lissauer, *Deut. med. Woch.*, 1908, p. 1335.

<sup>5</sup> *Proc. R. Soc. Med.*, Clinical Sect., 1908, p. 100.



preferred, the same doses being employed as are used for hypodermic injection. On the whole, we are not inclined to recommend this method of administration, and believe that the evidence adduced in its favour is fallacious.

Kapralik and Schrötter<sup>1</sup> find that tuberculin is also absorbed readily when administered by inhalation in the form of a spray; but there seems to be no advantage in this inconvenient mode of procedure.

A curious feature that is sometimes observed in the course of treating tubercular lesions with tuberculin is that while the original lesion is undergoing marked improvement, apparently as a result of the remedy, some new focus of tuberculosis may arise elsewhere, and may even run a rapidly progressive course. It is difficult to explain this occurrence in view of the general increase in resistance that should follow the appropriate use of tuberculin.

### SERUM THERAPEUTICS OF TUBERCULOSIS

Various attempts have been made to treat tuberculosis by an antitoxic serum on the lines of that used for diphtheria. To prepare the serum, horses or other animals are injected with gradually increasing doses of tuberculin or similar toxic products of the growth of the organism, and the serum obtained by subsequent bleeding of the animal is injected subcutaneously into the tuberculous individual.

**Maragliano's serum.**—For the preparation<sup>2</sup> of his serum, Maragliano uses two separate toxins—(1) a culture of the bacilli concentrated by heating on a water-bath at 100° C. for three or four days; and (2) a similar culture filtered through a Chamberland filter and concentrated *in vacuo* at a temperature of 30° C. A mixture, consisting of three parts of the former and one of the latter, is used to inoculate the horse, commencing with a dose of 2 mg. per kilogramme of body weight, and gradually

<sup>1</sup> *Wien. klin. Woch.*, 1904, No. 12, p. 583.

<sup>2</sup> Quoted from Nicholls, *Montreal Med. Journ.*, 1903, xxxii. 477.

increasing up to 40 mg. or 50 mg. The immunizing process lasts altogether about six months, a pause being made in the injections if the horse develops fever or other signs of illness. The serum is not drawn off for a period of three or four weeks after the injections have been stopped, until the urine of the animal ceases to contain toxic bodies. When it has been prepared, 1 c.c. of the serum will counteract the smallest dose of tuberculin capable of causing a reaction in an infected individual.

The serum is administered in doses of 1 c.c., which are injected on alternate days. The other means adapted to the cure of tuberculosis (open air and good feeding) are not omitted during the treatment with serum. The latter has, of course, no effect on the pyogenic organisms which secondarily infect the tuberculous individual (streptococci, etc.), but it is applicable to all cases, in whatever stage of the disease they may be, to combat the actual tubercle bacilli. The serum is said to be bactericidal as well as antitoxic, since if bacilli are kept for some days in the fluid they cease to cause infection when injected into animals, or to grow if planted on nutrient media.

Some statistics of the results obtained by the use of this serum are given by Mircoli.<sup>1</sup> In all, 2,899 patients came under consideration, and the uncomplicated cases may be thus tabulated:—

	Total	Cured	Im- proved	Station- ary	No effect
Circumscribed apyretic cases ...	250*	95	110	30	35
Circumscribed febrile cases ..	938	168	511	163	96
Diffuse tubercular bronchitis ..	665†	91	301	166	106
Advanced phthisis with cavities	712‡	39	281	102	240

\* (½) 270.

† (½) 664.

‡ (½) 662.

Such results are certainly noteworthy. Mircoli states that the improvement obtained is generally permanent,

<sup>1</sup> *Gaz. degli Ospedali*, Sept. 9, 1900; *Journ. Amer. Med. Assoc.*, 1900, ii. 887, 914.

relapses being infrequent—as if the organism, when it had once been assisted to defend itself against the tubercular parasite, were able to continue the struggle successfully for the future. The amount of complement present in the blood is said to be increased, and the antitoxic power is distinctly raised. This is not a mere passive immunity, due to the actual doses of antitoxin administered, as the increase is much greater than can be thus accounted for. On the other hand, Mircoli considers that the use of tuberculin adds to the amount of toxin present in the blood, and may actually overthrow an existing balance of immunity and cause the patient to succumb to the disease, which otherwise he might have successfully resisted.

Most other observers who have tried Maragliano's serum for the treatment of tuberculosis have failed to produce any marked improvement by its means.

**Marmorek's serum.**—Marmorek<sup>1</sup> claims to have isolated from the *B. tuberculosis* a special toxin, differing from tuberculin (this he considers only a subsidiary substance which aids in the production of the true poison), and by the inoculation of this toxin in horses has prepared a serum which acts as a protective to animals against tubercular infection. Marmorek has used the serum in cases of pulmonary tuberculosis and tubercular pleurisy, and claims good effects. He has also used it in tubercular meningitis, with some amelioration of symptoms, and thinks that it might prove curative of this condition if used early enough. The serum has been tried by a large number of observers with divergent results. Thus Ullmann<sup>2</sup> speaks of the serum as a specific remedy, superior to all other methods of treatment, and Monod,<sup>3</sup> Baer,<sup>4</sup> Schenker,<sup>5</sup>

<sup>1</sup> See *Lancet*, 1903, ii. 1470.

<sup>2</sup> *Wien. klin. Woch.*, 1906, No. 22.

<sup>3</sup> *Compt. Rend. Acad. Méd.*, Jan., 1907.

<sup>4</sup> *Münch. med. Woch.*, 1907, No. 24, p. 1670.

<sup>5</sup> *Ibid.*, 1907, p. 2125.

Roever,<sup>1</sup> and Thieme,<sup>2</sup> to quote only a few writers, are favourably impressed with its value. Glaessner<sup>3</sup> and Hoffa<sup>4</sup> found the remedy useful in surgical tuberculosis (joints, bones, etc.), and van Huellen<sup>5</sup> in abscesses and peritonitis. On the other hand, Krans<sup>6</sup> and Mann<sup>7</sup> found the serum useless in cases of pulmonary tuberculosis, and Stadelmann and Benfey<sup>8</sup> and Krokiewicz and Englander<sup>9</sup> believe it to be actually harmful.

Bosanquet and French<sup>10</sup> observed a rise in the opsonic index after rectal injections of the serum, but no improvement in the clinical features of their cases. In one instance in which the serum was administered subcutaneously a rapid fall in the opsonic index occurred, and the patient appeared to be harmed rather than benefited. This would seem to suggest that the serum contains some toxic body allied to tuberculin, rather than an antitoxin.

The evidence is at present too conflicting to allow a definite conclusion to be formulated as to the value of the serum. It would seem to merit further trial in cases of surgical tuberculosis, but it has proved disappointing in pulmonary disease. Rectal administration of the serum (5 c.c. daily) is to be preferred to the hypodermic method, but is disliked by patients. If the hypodermic method is employed, the doses should be smaller and the intervals between them longer.

**Other serums.**—Nicholls prepared an antitoxic serum by injecting goats with Koch's new tuberculin (T.R.).

<sup>1</sup> *Beitr. z. Klin. d. Tuberk.*, May 26, 1906.

<sup>2</sup> *Deut. med. Woch.*, 1908, No. 29.

<sup>3</sup> *Ibid.*, No. 16.

<sup>4</sup> *Berl. klin. Woch.*, 1906, No. 44.

<sup>5</sup> *Deut. Zeitschr. f. Chir.*, 1906, Nos. 1-3.

<sup>6</sup> *Zeitschr. f. Tuberk.*, 1905, vii., Hft. 3.

<sup>7</sup> *Wien. klin. Woch.*, 1906, No. 42.

<sup>8</sup> *Berl. klin. Woch.*, 1906, 93.

<sup>9</sup> *Wien. klin. Woch.*, 1906, No. 11. (For further literature see Catz, *Progrès Médical*, 1908, No. 26.)

<sup>10</sup> *Brit. Med. Journ.*, 1907, i. 862.

The injections were given subcutaneously in the neck, once a week, starting with doses of 0.0025 mg., and rising gradually to 15 mg. by the end of seven months. The serum thus prepared seemed to have some restraining power over the development of the disease in rabbits and guineapigs, but it could not be called curative.

Macfarland<sup>1</sup> injected an ass with tuberculin, and tried the serum obtained from it in 15 cases of tuberculosis, but without definite results.

Di Capra<sup>2</sup> finds diphtherial antitoxin useful in pulmonary tuberculosis. He states that under its influence fever diminishes, cough is relieved, and appetite improves (*see also* Normal Horse Serum, p. 234).

On the whole, we have to confess that at present it has not been found possible to produce a serum which will influence tuberculosis to an extent at all comparable with the effects of antitoxin in diphtheria. This want of success is probably owing to our inability to prepare an adequately strong solution of toxins of the tubercle bacillus. Much of the toxic matter of this organism seems to remain intracellular, and not to be given off into the culture-medium. The last word has not yet been said on the matter, and it is possible that more success will be obtained by further trials; but it is not in this direction that our hopes of combating the disease seem, at present, to point.

#### IMMUN KÖRPER (I.K.)

Spengler<sup>3</sup> has devised an immunizing preparation to which he gives the name "I.K." (Immun Körper). It is prepared from the red corpuscles of immunized animals, in which he believes the protective substances to be formed, and is administered hypodermically or by rubbing into the skin. It produces, according to its inventor's statement, improvement in breathing and in subjective

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1897, ii. 359.

<sup>2</sup> *Giorn. Internaz. delle Sci. Med.*, 1903, No. 2.

<sup>3</sup> *Deut. med. Woch.*, 1908, No. 38.

symptoms, and a disappearance of bacilli from the sputum. It can also be used for diagnostic purposes, as it produces a local reaction around tubercular lesions.

The majority of writers who have tried this preparation regard it as useless (Kapilowsky,<sup>1</sup> Roth,<sup>2</sup> Schaefer,<sup>3</sup> Baer<sup>4</sup>), but it is recommended by a few, such as Porter,<sup>5</sup> Hallos,<sup>6</sup> and Armstrong.<sup>7</sup> This last author describes the preparation as a clear, colourless liquid, with an acid reaction and a sp. gr. of 1004; it does not give a precipitate on boiling, but forms a ring with nitric acid and a precipitate with Millon's reagent. It is both lytic and antitoxic, and may exert a protective action in a dilution of 1 in 1,000 billion. *Credat Judæus!*

#### CONTRATOXIN

A substance called "Contratoxin," prepared by Melmarto, is said to consist of "various animal plasmas," including that of the lobster. It is recommended by the inventor for tuberculosis, as having a lytic action on tubercle bacilli as well as on staphylococci and streptococci. It was tried at Brompton Hospital by Dr. J. J. Perkins and Dr. A. C. Inman;<sup>8</sup> no good results were obtained. As the composition of the remedy is secret, its use is forbidden by the well-known professional rule, while its apparent ineffectiveness leaves no excuse for violating this canon.

#### ANTISTREPTOCOCCIC SERUM IN TUBERCULOSIS

It has already been pointed out that much of the destruction of the pulmonary tissue which takes place in cases

<sup>1</sup> *Abstr. in Zeitschr. f. Immunitätsforsch.*, 1910, p. 1200.

<sup>2</sup> *Münch. med. Woch.*, 1910, p. 296.

<sup>3</sup> *Zeitschr. f. Tuberk.*, 1910, xvi., Hft. 1.

<sup>4</sup> *Berl. klin. Woch.*, 1912, p. 208.

<sup>5</sup> *Med. Record*, 1911, lxxx. 349.

<sup>6</sup> *Virch. Arch.*, 1913, ccxiii. 380.

<sup>7</sup> "I.K. Therapy." 1914.

<sup>8</sup> Personal communication. We have not been able to find any literature on the subject.

of phthisis is due to a secondary infection of ulcerated surfaces by pyogenic bacteria, especially streptococci. The attempt has been made to combat these invaders by the use of antistreptococcic serum. Some results of this method of treatment are recorded by Bonney.<sup>1</sup> He selected cases in which large numbers of streptococci were to be found in the sputum. Other signs of infection with these organisms are to be seen in the occurrence of chills or irregular oscillation of the temperature, or in profuse sweating at night. Bonney used the serum in 26 cases, all of which were in an advanced stage of the disease and had failed to benefit by ordinary methods of treatment. As a result, 3 cases were apparently cured, and 4 others were set well on the way to recovery. Five cases improved distinctly, but not sufficiently to render recovery more than problematical; while 8 others exhibited temporary amelioration of symptoms, but the course of the disease was not checked. In 6 cases no definite results were obtained. Menzer<sup>2</sup> has also obtained good results with his serum in these cases, and considers that the inflammatory reaction which occurs, due to its action, may have a beneficial effect on the tubercular lesions, besides combating the streptococci.

#### GENERAL CONCLUSIONS

(1) **Tuberculin.**—A. The *original tuberculin* affords a valuable means of diagnosis when inoculated cutaneously or injected hypodermically. It is not infallible, the margin of error amounting to about 10 per cent. Calmette's modification of the original tuberculin is particularly useful as a diagnostic agent. It should not be used indiscriminately, but only in cases in which other means of diagnosis have been tried and failed, and in which the question of the existence of tuberculosis is of immediate importance to the patient.

<sup>1</sup> *Med. News*, July 13, 1903.

<sup>2</sup> *Münch. med. Woch.*, Oct. 27, 1903, p. 1877.



This form of tuberculin should not be used therapeutically, except perhaps in lupus.

B. The *new tuberculin* (T.R.) is of considerable value in the treatment of some cases of lupus vulgaris, especially those in which the disease has involved parts inaccessible to direct surgical or photo-therapeutic measures. It may do good also in some cases of laryngeal tuberculosis. The best results from its use are obtained in tuberculosis of the genito-urinary tract, of the peritoneum, of the eye, and of bones and joints. Early tuberculous adenitis also yields to tuberculin T.R., but enlarged glands of old standing do not undergo any marked alteration. It is contraindicated in tubercular meningitis.

This form of tuberculin may perhaps be of value in selected cases of pulmonary tuberculosis. It should be used, if at all, along with ordinary treatment by open air and increased feeding in patients who are practically free from fever, especially in cases which appear to be making little progress by hygienic measures alone.

(2) **Agglutination test.**—This method of diagnosis, as at present carried out, is complicated and unreliable. It is of no practical value for the diagnosis of phthisis. It may, perhaps, be of use for distinguishing between tubercular ascites and other collections of fluid in the abdomen.

(3) **Serum treatment.**—A. Treatment of tuberculosis by the serum of immunized animals is at present unsatisfactory. Maragliano's and Marmorek's serums may possibly be of some use, but the evidence is not conclusive.

B. In cases of pulmonary tuberculosis accompanied by the presence of large quantities of streptococci in the sputum, treatment with antistreptococcic serum appears to have given good results, and is certainly worthy of trial.

(4) **Protective vaccination.**—It is possible to render animals immune to tuberculosis by vaccination with attenuated bacilli. As to the announcement by Maragliano, and by von Behring and others, of a means of vaccinating human beings against the disease, no sufficient data are available.

## CHAPTER XVII

### LEPROSY; STREPTOTRICHOSIS; RINGWORM

#### LEPROSY

**Etiology.**—The *Bacillus lepræ* was discovered by Hansen in 1886. Attempts to cultivate it artificially have generally failed, but one or two successful cultures have been reported. The lower animals appear to be insusceptible to this organism.

**Complement-fixation.**—Biehlen and Eliasberg<sup>1</sup> state that they obtained fixation of complement with the serum of lepers, using as antigen a solution of leprous nodules in antiformin. Duval<sup>2</sup> makes a similar statement, his antigen being a culture of the bacillus; but assertions as to the cultivation of *B. lepræ* are to be received with caution.

**Serum treatment.**—Carrasquilla<sup>3</sup> endeavoured to prepare an antidotal serum by injecting the blood of lepers into asses and young horses. The resulting serum is probably quite inert. Sugai, Mabuchi, and Mononobe<sup>4</sup> prepared a serum by injecting goats with an emulsion of leprous tissue. They believed that they saw beneficial results in patients treated with this serum, the rash diminishing and nodules softening.

**Non-specific treatment.**—Attempts have been made to treat the disease with *antivenene*, and good results are

<sup>1</sup> *Lepra Bib. Internat.*, 1910, ix. 207.

<sup>2</sup> *Med. Record*, 1911, lxxix. 177.

<sup>3</sup> *Deut. med. Woch.*, 1897.

<sup>4</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1910, p. 1157.

claimed for this method by Dyer<sup>1</sup> (5 cases; 2 cured, 3 improved). Injections of *tuberculin* have also been tried, and have apparently resulted in some softening of leprous nodules, but no permanent good has been effected. Abraham<sup>2</sup> states that "fresh nodules crop up, and the patients are generally no better off after treatment than before." The disease runs a very chronic course, with intervals of improvement or cessation of advance. Hence many remedies have been advocated as cures, the natural remissions being attributed to their action. There is, unfortunately, no greater reason to think that any serum is a reliable remedy than to believe in the methods of drug treatment which have been advocated.

**Vaccine treatment.**—Deycke and Reschad<sup>3</sup> isolated a streptothrix from cases of leprosy and used it as a vaccine: they observed good results. They have also separated from the organisms a fatty substance to which they give the name of "nastin"; this they use in the form of an oily solution for injection. They suggest that it might also be of use in tuberculosis. Different strengths are obtainable, labelled B 0, B 1, B 2. Perper, using the two stronger solutions, claims that he cured 2 cases out of 5 treated. Kupffer<sup>4</sup> reported very favourably on the results obtained from the use of this agent, although in many of his cases chaulmoogra oil was also administered. At a discussion at the International Leprosy Congress, 1909, very different views were expressed as to the value of nastin, and we are not inclined on theoretical grounds to regard it as a likely specific for the disease.

For reasons we have already given we venture to express some scepticism as to the claim of Roos<sup>5</sup> to have

<sup>1</sup> *New Orleans Med. and Surg. Journ.*, Oct., 1897.

<sup>2</sup> Art. "Leprosy," in Allbutt's "System of Medicine," ii. 78.

<sup>3</sup> *Deut. med. Woch.*, 1907, p. 89.

<sup>4</sup> *Lepra Bib. Internat.*, 1909, viii., Part 3.

<sup>5</sup> *Ibid.*, 1912, xii. 125.

benefited 12 cases of leprosy by the use of a vaccine of *B. lepræ*.

### STREPTOTRICHOSIS

Wynn<sup>1</sup> has recorded a case of actinomycosis of the lung successfully treated with a vaccine. The patient was a boy of 14 years, and the dose used was 0·001 mg. of the solid substance of the organism isolated from the pus and grown on agar ("actinomycotin"). The authors also have had very satisfactory results in a series of cases not yet recorded.

**Agglutination test.**—Widal and Abrami<sup>2</sup> found in cases of sporotrichosis that the spores (not the mycelium) of the fungus *Sporotrichum beurmanni* were agglutinated by the patient's serum, but that this reaction was not strictly specific, a "group reaction" occurring with serum of patients suffering from actinomycosis or from thrush, though not with that derived from cases of tinea or favus. This agglutination reaction with sporotrichial spores has been used for the diagnosis of actinomycosis, the reaction occurring in dilutions of 1 : 30, or even 1 : 300.

### RINGWORM

Bruck and Kusonoki,<sup>3</sup> who cultivated the trichophyton fungus on a special medium, filtered and sterilized the liquid, and put it up for use with a little phenol as a preservative. They first employed this so-called "trichophytin" as a diagnostic test, as it may give a reaction, analogous to the tuberculin reaction, when injected into patients affected with ringworm. They found also that it had some curative effect. Stein<sup>4</sup> finds it useful in kerion and sycosis, the best species of the hyphomycetes for such use being *Achorion*

<sup>1</sup> *Brit. Med. Journ.*, 1908, i. 554.

<sup>2</sup> Widal and Abrami, *Semaine Méd.*, 1908, p. 309; Corgon and Gougliot, *ibid.*, 1910, p. 117; Widal, *ibid.*, 1910, p. 238; Jeanselme, *ibid.*, 1910, p. 308.

<sup>3</sup> *Dent. med. Woch.*, June 15, 1911.

<sup>4</sup> *Wien. klin. Woch.*, 1912, p. 1817.

*quinckeianum*. He also uses an ointment of trichophytin for superficial cases which fail to give a reaction to hypodermic injections. Lombardo<sup>1</sup> finds that trichophyton extracts are of no value in the treatment of chronic cases of ringworm.

<sup>1</sup> *Giorn. Ital. d. Mal. Ven.*, 1912, liii., No. 2.

## CHAPTER XVIII

### AFFECTIONS DUE TO STREPTOCOCCI

#### STREPTOCOCCIC SEPTICÆMIA, RHEUMATISM, CHOREA, SCARLATINA

**Nature of the organisms.** — Much controversy has centred round the question of the identity or diversity of the streptococci found in different conditions. Some writers<sup>1</sup> have enumerated eighteen different (?) species. An attempt was at one time made to differentiate them into "short" and "long" varieties, viz. those which formed chains consisting of a large number of separate cocci, and those which occurred in smaller groups; but this classification is untenable, as the same coccus may form short or long chains according to the circumstances of its environment. Within the body of an infected animal these organisms generally occur in pairs, or as separate units, only showing chain-formation in artificial media. On the other hand, the pneumococcus (*Diplococcus pneumoniae*) frequently takes the form of chains of cocci when grown outside the body. There appears to be no essential difference with regard to chain-formation between streptococci and many so-called diplococci.

Many observers hold that all the streptococci found in different pathological conditions are in reality the same organism, modified in some of its characteristics by circumstances. A strong upholder of this view is Marmorek,<sup>2</sup> who has examined organisms derived from

<sup>1</sup> Andrewes and Horder, *Lancet*, 1906, i. 1245.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, March, 1902, *et passim*.

erysipelas, puerperal fever, scarlatina, pustules and boils, and sore throats, and finds that all of them produce the same poison, and that all are antagonized by an antitoxic serum prepared from cultures of any one of them. Aronson<sup>1</sup> is of the same opinion; he finds that a horse immunized against one variety is resistant to all.

On the other hand, Meyer,<sup>2</sup> using the method of agglutination, believes that there are two different species: (1) the pyogenic organism, met with in erysipelas, suppuration, etc.; and (2) a streptococcus met with in cases of angina (sore throat). Andrewes and Horder<sup>3</sup> distinguished five different species, and Foulerton<sup>4</sup> also believes in the existence of more than one species, these writers basing their views upon the changes produced in solutions of various carbohydrates.

At present the question of the unity or diversity of the streptococci cannot be answered with certainty. It is clearly of the greatest importance. For the moment we have these two clinical facts strongly before us—viz. that since the introduction of "polyvalent" antistreptococcic serum there has been a marked improvement in therapeutic value, and further, that frequently, when the serum prepared by one manufacturer has proved entirely without effect, the employment of antistreptococcic serum from another maker has been immediately followed by a complete amelioration of the patient's condition. It seems difficult to believe that the pneumococcus, which has, besides other peculiarities, the property of forming a capsule, is not a separate species of organism. Other diseases in which chain-cocci have been met with are rheumatism, chorea, and scarlatina. We may consider the questions of serum treatment connected with these organisms under the follow-

<sup>1</sup> *Deut. med. Woch.*, 1903, June 18, p. 439.

<sup>2</sup> *Ibid.*, 1902, No. 42. For experiments on agglutination of streptococci, see also Zelenski, *Wien. klin. Woch.*, 1904, p. 406.

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *Lancet*, 1904, ii. 1828.



ing heads : (1) Septicæmia and Erysipelas ; (2) Rheumatism ; (3) Scarlatina.

## 1. SEPTICÆMIA AND ERYSIPELAS

**Streptococcus pyogenes.**—This organism was first observed by Ogston in 1881, and described by Rosenbach in 1884. It has been found in cases of spreading cellulitis and pyæmia, in some instances of malignant endocarditis, in puerperal sepsis, in erysipelas, in the pyogenic affections complicating acute infectious diseases (small-pox, enteric fever, etc.), in some membranous sore-throats, in a variety of intestinal lesions, and in the cutaneous affections known as ecthyma and impetigo contagiosa. Marmorek<sup>1</sup> has prepared a special medium for growing streptococci, consisting of ordinary broth, peptonized meat, leucine, and glyocol. He has also grown them in a mixture of broth and human serum, and in broth mixed with serous fluid from the pleura or peritoneum. By cultivating them in this way and alternately passing them through rabbits he has succeeded in producing bacteria of such virulence that 0·000000000001 c.c. of the culture will inevitably kill a rabbit. This quantity is calculated to contain, on an average, one single streptococcus.

If any pathogenic streptococcus is grown in a fluid medium until growth ceases, no other strain of these organisms will subsequently flourish in that fluid without addition of further nutrient material. This test is used by Marmorek to prove the identity of all the streptococci.

**Toxins.**—Marmorek finds that all streptococci, whatever their origin, manufacture the same toxin. It is of the nature of a diastase, and its activity is destroyed at a temperature of 70° C. In addition to the diastatic ferment, streptococci give rise to a poisonous substance which has hæmolytic powers ; this has been called “streptocolysin,” and is said to possess a toxophore and a haptophore group.<sup>2</sup>

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1895, ix. 593 ; also 1902, xvi. 169.

<sup>2</sup> Ruediger, *Journ. of the Amer. Med. Assoc.*, Oct. 17, 1903.

It is destroyed at a temperature of 70° C. Its presence accounts for the petechial eruptions, and perhaps for the jaundice, so often met with in septic conditions.

#### ANTISTREPTOCOCCIC SERUM

The serum originally introduced by Marmorek was prepared by inoculating horses with the virulent organisms obtained in the way previously recorded. Living cultures are more efficacious in the formation of a potent serum than are dead organisms. Very small doses are used for the first injections, which are gradually raised as the animal becomes more resistant: the final dose may reach 600 c.c. of a virulent culture. The process extends over a year. Each injection must be sufficient to produce a distinct reaction; and the blood of the horse is withdrawn four weeks after the last injection. Marmorek finds that it is impossible to produce an effective serum by means of the toxins of the cocci alone; apparently these, as obtained in artificial media, are not strong enough. He considers that his serum has a weak antitoxic power; it is chiefly bactericidal. Raw,<sup>1</sup> on the other hand, maintains that the Paris serum is mainly antitoxic.

Many authorities, however, hold that the passage of streptococci through animals reduces their virulence for man, and that a fundamental error is inherent in Marmorek's method of preparing his serum. Of the different brands of serum now in use, those of Marmorek and Denys are made from organisms passed through animals, and those of Tavel, Krumbein, Moser and Menzer from streptococci derived from human sources. Aronson combines both kinds. Experiments by Meyer and by Sommerfeld appear to show that of these serums Aronson's is the most effective.<sup>2</sup>

**Value of antistreptococcic serum.**—A few general

<sup>1</sup> *Lancet*, 1898, ii.

<sup>2</sup> *Zeitschr. f. klin. Med.*, 1900; *Centralbl. f. Bakt.*, I. Orig., 1903, xxxiii. 722.

considerations as to the use of antistreptococcic serum may be set out at this point. In the first place, it must be remembered that cases of infection with pyogenic organisms differ from one another in severity almost more than do attacks of any other kind of disease. It is, therefore, practically impossible to form any estimate of the mortality of such cases from statistics, and equally impracticable to apply the statistical method to recorded cases of cure or failure. In cases which appear hopeless, recovery may take place in a most marvellous manner without the use of any specific remedy. If such a "cure" is effected in a case treated with serum, there is naturally a tendency to ascribe the good result to the serum. On the other hand, there are many instances in which the infection is so intense and so rapid in its onset that it would be hopeless to expect any antibacterial serum to prove efficacious. Thus only a very large collection of cases would serve as a basis for statistical calculation, and this the nature of the disease makes it specially difficult to procure, as most instances of invasion by pyogenic organisms may be described as "accidental"; that is to say, they are sporadic in occurrence and due to inoculation of cocci in wounds, etc., while the state of health of the individual attacked appears to play a greater part in the process than in most other diseases. We are thus deprived of the aid afforded by the epidemic occurrence of disease, in which large numbers of cases are met with under very similar circumstances, are often treated in special hospitals, and are readily adapted for collection and tabulation. Finally, many of the series of cases reported are of a very heterogeneous nature, embracing, for example, puerperal fever, erysipelas, and cellulitis, the writer applying the results obtained in all of these together to establish the value of the serum in a general way. It will be best briefly to consider the opinions that have been expressed as to serum treatment in each of these conditions separately.

The exact mode of action of antistreptococcic serum is not satisfactorily determined. It is not bacteriolytic *in*

*vitro*. Menzer<sup>1</sup> believes that it acts by inducing phagocytosis (opsonically), but Reisch<sup>2</sup> denies this in the case of Aronson's serum.

**Puerperal fever.**—A special committee was appointed by the American Gynecological Society to consider the value of antistreptococcic serum in puerperal sepsis. The committee reported<sup>3</sup> that they had collected records of 352 cases treated with serum, among which the mortality was 20·74 per cent., whereas among all cases of the disease not so treated the total death-rate worked out at 5 per cent. It would seem on the face of this report that the committee must have concluded that the use of the serum had increased the mortality of puerperal fever by about 15 per cent.—a fair instance of proving too much, since no one can reasonably maintain that deleterious effects are often due to the serum, and this the committee themselves admitted; the most that can be said against it is that it is ineffectual. Hence the only conclusion that can be fairly drawn from the above figures is that the statistical method is untrustworthy in this instance. The report of the committee is valuable as affording a very complete bibliography of cases recorded up to the time of its appearance.

Savor<sup>4</sup> records his results in 16 cases: in 6 no good effects were seen, in 5 the value of the remedy was doubtful, in the remaining 5 good results were obtained.

Blumberg<sup>5</sup> tried the serum in 12 cases, all of them severe, in 9 of which a bacteriological examination was made of the lochia. Two cases showed anaerobic diplococci; no good effects from serum. Four cases showed mixed infection, some streptococci; two died, one improved after injection. Two cases showed sterile lochia; both

<sup>1</sup> *Münch. med. Woch.*, June 23 and 30, 1903. Cf. Zangemeister, *Deut. med. Woch.*, July 5, 1906.

<sup>2</sup> Abstr. in *Centralbl. f. Bakt.*, I. Ref., 1906, xxxviii. 72.

<sup>3</sup> *American Journ. of Obstetrics*, 1899, xl.

<sup>4</sup> Quoted by Blumberg (*see below*).

<sup>5</sup> *Berlin. klin. Woch.*, 1901, No. 5, p. 132.

recovered, a fall of temperature occurring after injection. Two cases showed pure streptococcal infection; both of these recovered. Three cases (lochia not examined); two showed fall of temperature after injection (one already convalescent).

Blumberg is favourably impressed as to the value of the serum. Peham<sup>1</sup> also records good results in cases of pure streptococcic infection; in mixed infections and cases due to other organisms no good results can be expected. Burkard<sup>2</sup> treated 29 cases of pure streptococcic infection without a death. Falkner<sup>3</sup> records 83 cases with 14 deaths, and thinks the serum useful; and Bumm<sup>4</sup> also noted favourable results, using Aronson's serum. Pilcer and Ebersen<sup>5</sup> speak favourably of Marmorek's serum as an adjuvant to other methods of treatment; but McMurtry<sup>6</sup> believes all serum treatment of puerperal septicæmia to be quite useless. Many isolated cases of benefit attributed to the remedy in puerperal sepsis are recorded, but on the whole the results seem to have been disappointing. We can only conclude that further observation is needed to establish its true position, but that it is advisable to give the serum a trial in cases in which streptococci are found.

**Cutaneous test.**—Köhler<sup>7</sup> describes a cutaneous test for this condition. A culture is prepared of many strains of streptococci, the organisms are killed, and the fluid filtered off. The fluid is rubbed into the skin as in von Pirquet's test, and, if streptococci are present in the blood, an inflammatory swelling ensues at the point of application and reaches its maximum in about eight hours. The reaction may fail to appear if the patient is *in extremis*.

<sup>1</sup> *Wien. klin. Woch.*, 1904, p. 405.

<sup>2</sup> *Arch. f. Gynäk.*, Bd. lxxix., Heft 3.

<sup>3</sup> *Wien. klin. Woch.*, 1907, p. 1582.

<sup>4</sup> *Berlin. klin. Woch.*, Oct. 31, 1904.

<sup>5</sup> *Therapeut. Monats.*, 1904, p. 509.

<sup>6</sup> *Brit. Med. Journ.*, 1906, ii. 1207.

<sup>7</sup> *Monatsh. f. Geburtsh. u. Gynäk.*, 1912, xxxv., Heft 2.

**Erysipelas.**—Marmorek states that he treated 423 cases of erysipelas with his serum, with good results, the mortality being 3·87 per cent. Denys<sup>1</sup> reports good effects from local injections of the serum into the neighbourhood of the affected area. He gives four injections of 0·25 c.c. around the lesion. Mayer and Michaelis,<sup>2</sup> and Ayer,<sup>3</sup> noted improvement in cases treated with serum; and we have ourselves seen apparently good results in a few instances.

Welz<sup>4</sup> finds good results in about 50 per cent. of all cases from the use of antistreptococcus serum.

The serum of convalescents has been tried by Fornaca,<sup>5</sup> with apparent benefit, but does not seem to be a practicable remedy. Diphtherial antitoxin has also been recommended.

**Cellulitis and septicæmia.**—Thomas<sup>6</sup> records a series of 15 cases of sepsis successfully treated with the serum; he administers doses of 30 c.c. Good results are reported by Marmorek and others. One of the present writers had a personal experience of the use of serum in his own case, when he was suffering from spreading cellulitis due to a poisoned post-mortem wound. The subjective effect was certainly good, the feeling of illness and mental disturbance lessening concomitantly with its use. The temperature also fell gradually, but it is impossible to be sure whether this was attributable to the serum or to the surgical measures adopted. In this case the serum was administered in doses of 10 c.c. every four hours for several days. No ill effects were noticeable, but for some time after the injections there was very intense itching at the sites of injection, and faint lines of pigmentation marked the tracks made by the needle.

**Ulcerative endocarditis.**—Some good results have

<sup>1</sup> *Semaine Méd.*, 1901, p. 40.

<sup>2</sup> *Berlin. klin. Woch.*, 1903, p. 377.

<sup>3</sup> *Med. Record*, Aug. 26, 1905.

<sup>4</sup> *Therapeut. Monats.*, 1913, p. 273.

<sup>5</sup> *Polyclin.*, July, 1905.

<sup>6</sup> *Journ. of American Med. Assoc.*, Feb. 18, 1899.

been obtained in this disease by the use of the serum. Thus, 2 cases are recorded by Mitchell Bruce<sup>1</sup> in which apparent cure resulted. In one of these a certain brand of serum was found ineffectual, only a temporary fall of the fever being observed; but on using a serum prepared by a different maker, permanent improvement was effected.

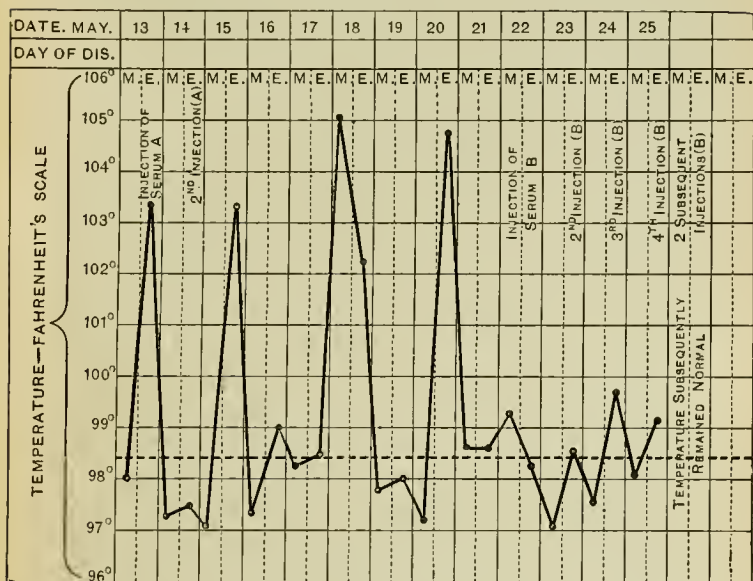


CHART 3.—ILLUSTRATING THE EFFICACY OF A SECOND BRAND OF ANTISTREPTOCOCCIC SERUM AFTER FAILURE OF THE FIRST.

This case, which came under the notice of the present writers (*see* Chart 3), is instructive as illustrating one reason for the want of success which has often been experienced with this serum. Such failure may be due, apparently, to the existence of various strains of the cocci: each strain gives rise to a serum which is effectual against that particular variety, but which does not equally lead to the destruction of

<sup>1</sup> *Lancet*, 1898, ii. 515,



other strains of streptococci derived from different sources (*see also* Staphylococci, p. 383).

Ogle<sup>1</sup> also speaks favourably of the use of the serum in malignant endocarditis, and Dyce Duckworth<sup>2</sup> records a case in which *rectal* administration of the serum apparently effected a cure. Raw,<sup>3</sup> again, saw good results from this method of treatment, 20 c.c. of serum being given with 40 c.c. of hot saline solution. Ward<sup>4</sup> collected a total of 25 cases, of which 8 were cured and 3 temporarily improved.

**Pernicious anæmia.**—Hunter,<sup>5</sup> relying on his discovery of streptococci in cases of pernicious anæmia, treated some patients suffering from this disease with antistreptococcic serum. He considered that good results were produced. In one case of this disorder, which was admitted to Charing Cross Hospital, the use of the serum was followed by collapse and death, which appeared to be due to the injection, and may have been analogous to the few recorded instances of death occurring after diphtherial antitoxin. It was, however, impossible to be certain that the remedy was actually accountable for the death of the patient, as pernicious anæmia is a disease which causes profound degeneration of the cardiac muscle, and it is difficult to be sure of the exact degree of this myocardial disease in an individual case. Sudden death may occur from asystole at any time. The rapid onset of delirium and coma after the injection was, however, remarkable.

**Gangrenous stomatitis.**—Cahall<sup>6</sup> reports a case of this affection successfully treated with the serum. Diphtherial antitoxin may be useful in cases of noma due to *B. diphtherice*.

<sup>1</sup> *Lancet*, 1903, i. 720.

<sup>2</sup> *Brit. Med. Journ.*, 1903, i. 1195.

<sup>3</sup> *Lancet*, 1906, i. 1103. Cf. Fenwick and Parkinson, *ibid.*, 1244.

<sup>4</sup> *Albany Med. Annals*, Oct., 1903, p. 515.

<sup>5</sup> *Lancet*, 1900, i. 374.

<sup>6</sup> *Philadelphia Med. Journ.*, Feb. 17, 1900,

**Purpura hæmorrhagica.**—Coutts<sup>1</sup> has successfully treated 2 cases of this disease by rectal injections of antistreptococcic serum. He expresses, however, some doubt whether normal (horse) serum would not have been equally effective (*see* p. 434).

The use of antistreptococcic serum in small-pox and in phthisis is alluded to elsewhere (*see* pp. 199 and 342).

**Local collections of pus.**—In cases in which actual collections of pus have formed, the use of the serum can do no good, so far as the local lesion is concerned. Surgical measures for the evacuation of the pus must be adopted. Subsequently the serum may perhaps prevent the formation of other foci of suppuration, or the death of the patient from septicæmia. Menzer considers that, if pus be present, the serum may even do mischief, as it may cause a breaking-up of a large number of the streptococci, and so produce an intoxication of the whole system by absorption of poisonous products. This is possibly true, if local surgical measures are neglected.

**Ill effects of the serum.**—Horse's serum being the basis of antistreptococcic serum, the same by-effects may occur after its employment as after diphtherial antitoxin, viz. erythematous and urticarial eruptions and pains in the joints. Allusion has just been made to one fatal case that was apparently due to the serum.

**Diphtherial antitoxin in septic conditions.**—Diphtherial antitoxin has now been tried in a large number of different conditions besides diphtheria, and benefit has been ascribed to its use. Paton<sup>2</sup> considers that it is almost specific for septic conditions generally. He concludes (1) that it is specific for all affections due to streptococci and staphylococci, and for simple traumatic inflammations; (2) that it acts as an absorbent for inflammatory tissues and for effused blood; (3) that it has an influence on the coagulability of the blood; and

<sup>1</sup> *W. London Med. Journ.*, 1908, p. 286.

<sup>2</sup> *Australasian Med. Gaz.*, Feb. 20, 1902,

that (4) it acts beneficially in depressed nervous conditions (which may perhaps be due to septic or autotoxic conditions).

Paton considers that the serum acts well when it is given by the mouth, and he adopts this method of administration. He gives the following formula: Diphtherial antitoxin, 3,000 units; trag. carmin., a sufficiency; water to 2 ounces. One-fourth part of this (750 units) is given night and morning, or every four hours. In erysipelas the dose is administered every eight hours. In acute peritonitis or perityphlitis it is given at once, and repeated in two hours' time; then, again, every four to six hours. The serum may cause slight renal irritation or cutaneous eruptions, but these effects are unimportant.

More recently Paton appears to state that the amount of units of antitoxin given is immaterial, and that the normal serum of the horse or ox is almost equally effective (*see p. 434*), the process of immunizing the animal against diphtherial toxins only calling out in greater amount some body always present in the animal's serum, which stimulates the cells of the human body to resist microbial attack.<sup>1</sup>

In the present state of our knowledge it appears difficult to take very seriously any claims as to the value of an antitoxin in other diseases than that for which it is prepared. It may possibly produce some leucocytosis, and thus be beneficial, but it is at least as probable that the cures attributed to it are instances of the fallacy of mistaking *post hoc* for *propter hoc*.

**Conclusions.**—It is impossible to resist the conclusion that on the whole the use of antistreptococcic serum has been disappointing. This may be due to many causes. In the first place, the existence of several strains of the cocci, which react differently to a given serum, introduces a constant source of failure, especially if only one brand of serum is available for use. In cases which are sufficiently chronic to

<sup>1</sup> "New Serumtherapy." London, 1906.

admit of the trial of a second brand in the event of primary failure, this course should be adopted, and may prove successful.

In the second place, all antibacterial serums appear to keep badly, quickly losing their bacteriolytic power. Hence only freshly prepared serum should be employed. Many of the serums on the market have at one time or another been found to be inert. Some of the disappointments recorded may have been due to neglect to secure a freshly prepared serum.

Again, many cases of sepsis are not due to streptococci alone, but are complicated by the presence of other germs. Although the antistreptococcic serum may be able to counteract the former organisms, yet the others may have gained so firm a footing as to prove fatal to the patient.

Finally, there can be little doubt that, until lately, recourse was seldom had to serum treatment till the disease was too far advanced. Owing to the difficulty of procuring the serum and the expense involved, the remedy was only administered as a last resource. We have seen that in the case of all serums the most important condition for success is early use. Now that a more plentiful supply of serum is obtainable, and the medical profession is becoming better acquainted with the value of these remedies, it may be hoped that better results will be recorded.

We cannot doubt that in a certain proportion, at all events, of the cases recorded, the serum has acted most beneficially. It is impossible to know beforehand, on any general grounds, which cases will react to the remedy and which will show no improvement. It would seem, therefore, advisable, in all cases in which infection by streptococci threatens to become generalized (septicæmic), to have recourse to the serum at as early a stage as possible. No harm is likely to result in any case, and a fatal septicæmia may be warded off.

No assistance can be expected from antistreptococcic serum in cases due to infection by other pyogenic organisms

—staphylococci, *B. pyocyaneus*, etc. It is, therefore, advisable to ascertain, if possible, whether any individual case is due to the presence of streptococci, before using the serum. For this purpose from 5 to 10 cubic centimetres of blood should be withdrawn, preferably from a vein in the arm, by means of a sterile syringe, and distributed amongst several tubes or flasks of suitable fluid culture-medium; or the pus from any local lesion which is available may be examined for the cocci; or the streptococci may be sought for in the urine, by which channel they are often excreted. In cases, however, in which a bacteriological examination is not immediately possible, it is preferable to inject the serum at once, if the symptoms are severe and point to streptococcic infection, rather than wait any considerable time for the bacteriological report.

**Summary of treatment with antistreptococcic serum.**—After adopting such surgical measures as are indicated—

1. Inject 20 c.c. of polyvalent antistreptococcus serum, at least half intravenously.

2. If marked improvement occur, repeat the dose in twenty-four hours.

3. If no obvious improvement be noted, administer a similar dose of polyvalent serum prepared by another manufacturer.

4. If there be still no improvement, try a third brand.

#### VACCINE TREATMENT

Recently, attempts have been made to treat affections due to streptococci by means of vaccines consisting of dead organisms. It does not seem probable that much good can result from this procedure in cases of **general septicæmia**, though Sutcliffe and Baily<sup>1</sup> attribute the cure of one such case to the use of a vaccine, the dose rising from 10,000,000 to 50,000,000 streptococci.

<sup>1</sup> *Lancet*, 1907, ii. 367.

Watters and Eaton<sup>1</sup> speak highly of polyvalent vaccines. Western<sup>2</sup> states that while the mortality of all cases in which living organisms are found in the blood is from 85 to 95 per cent., after the use of vaccines it is reduced to 55 per cent.

Barr and Douglas<sup>3</sup> record the apparent cure of a case of **ulcerative endocarditis** by means of a vaccine prepared from the actual organism derived from the patient. Horder<sup>4</sup> also tried this method, but without success. We ourselves have had cases in which the cure has apparently depended upon the vaccine used; but on the other hand we have had many disappointing cases where the vaccine has failed to avert the fatal termination.

Craig<sup>5</sup> states that he has collected from the literature 29 cases of acute ulcerative endocarditis treated with vaccines: of these 13 were cured and 8 improved. It may be well to repeat here that unsuccessful cases are not usually recorded.

In localized infections with streptococci good may be effected. Wynkoop<sup>6</sup> records a case in which streptococci were found in the cerebro-spinal fluid, and cure resulted after the use of a vaccine.

Still<sup>7</sup> records 10 cases of **erysipelas** in infants treated with vaccines, with 1 death. In 7 cases a streptococcic vaccine alone was used; in 1 a mixed vaccine of streptococci, staphylococci, and *Bacillus coli*; and in 2 a "mixed phylacogen." Weinstein<sup>8</sup> cured postoperative fistulæ by the use of vaccines.

In a case of **appendicitis** in which œdema occurred

<sup>1</sup> *Boston Med. and Surg. Journ.*, April 13, 1911.

<sup>2</sup> *Lancet*, 1912, i. 351.

<sup>3</sup> *Ibid.*, 1907, i. 499.

<sup>4</sup> *Ibid.*, July 16, 1904.

<sup>5</sup> *Med. Record*, 1911, lxxx. 1015.

<sup>6</sup> *N.Y. Med. Journ.*, July 9, 1910.

<sup>7</sup> *Med. Record*, 1913, lxxxiii. 573.

<sup>8</sup> *Berlin, klin. Woch.*, 1906, No. 39,

after operation in the neighbourhood of the wound, Hawkins and Corner<sup>1</sup> successfully employed inoculations of a vaccine of *Streptococcus fecalis*, the organism present. Inoculations of a vaccine of *B. coli* were employed simultaneously, but Wright, who carried out the opsonic and bacteriological investigations in the case, considered that this organism played at most a subsidiary part.

**General conclusions.**—From what has already been said regarding the various species of streptococci, it would appear probable that stock vaccines would be useless in the treatment of generalized streptococcic infections; and in practice this is found to be so—the vaccine employed must be prepared from that species actually infecting the patient. Further, as time is of such great importance, every device that will shorten the period spent in preparing the vaccine must be adopted. Consequently, cultivations from septic material should be planted directly on to blood-agar plates, and, if these are carefully observed during incubation, the vaccine can often be completed in 24 hours when the streptococcus is present in pure culture; but even when several different kinds of bacteria are present it will usually be found possible to establish pure cultures of the streptococcus after at most 12 hours; and cultivations 12 to 18 hours old will suffice to prepare a few doses of vaccine, so that treatment can often be commenced within 36 hours. It is possible to adjust the dosage of vaccine without estimating the movements of the opsonic index, and in the absence of this exact guide the temperature and pulse must be carefully watched, for it may be taken as a general, though not invariable, rule that high temperature and rapid feeble pulse coincide with a low index. The object to be aimed at is the elimination of the negative phase and the production of a series of short positive phases gradually increasing in duration. Consequently, the initial dose should be small—from 0·5

<sup>1</sup> *Brit. Med. Journ.*, 1908, ii, 782.



to 2·5 millions. With such a dose the negative phase is short, sometimes absent; consequently a fall in temperature usually occurs in about 12 hours. But the succeeding positive phase is also short, and it is often necessary to repeat the dose in 36 to 48 hours. Subsequent doses, gradually increased in size, are generally needed at gradually increasing intervals, until convalescence is established.

## 2. RHEUMATISM AND CHOREA

**Etiology.**—The causation of **rheumatic fever**, and of the chronic affections of the joints generally described as rheumatic, is not definitely known.

Many observers have found micro-organisms in the blood and synovial fluid of patients suffering from acute rheumatism. A large number of different bacteria have been described in this connection—staphylococci, streptococci, diplococci, and bacilli. For practical purposes the question of the microbial origin of the disease is at present associated with the claims of an organism first discovered by Apert and Triboulet (1898), and subsequently investigated by Wassermann, and in this country by Poynton and Paine.

Walker calls the organism the *Micrococcus rheumaticus*. It would be preferable, perhaps, to call it a streptococcus, as it appears to belong to this group.

With regard to the specific nature of this organism, no certain verdict has been pronounced as yet. The opinions of some authorities as to the unity of the streptococci have already been mentioned. On the other hand, it is difficult to believe that so distinct a clinical disease as rheumatism can be due to the same cocci which produce suppuration. If the rheumatic cocci be merely an attenuated form of these, it is curious that suppuration is practically never seen in rheumatic joints, as it might have been expected that the organisms would not infrequently gain increased virulence in susceptible persons. There is

no reason to believe that rheumatic subjects are in any way refractory to suppurative lesions. Walker, moreover, finds that the rheumatic cocci will grow in a filtered culture-fluid in which other streptococci have been grown and have died out. This, which has been alluded to as Marmorek's test for the unity of the streptococci, appears to show that the *Streptococcus rheumaticus* is distinct from ordinary pyogenic organisms, whilst many observers consider that the organism isolated from the joints is a variety of the pneumococcus.

It must be mentioned that some observers have failed altogether to find micro-organisms in cases of acute rheumatism. Thus, McCrae<sup>1</sup> examined 270 cases with practically negative results; and Triboulet,<sup>2</sup> finding organisms in some cases and not in others, considers that the sterile cases represent the true disease, while those due to organisms only simulate rheumatism. In any case there will be found but few bacteriologists willing to accept the *Streptococcus rheumaticus* as a specific micro-organism, or as the specific cause of the disease.

**Menzer's serum.**—The serum used by Menzer is prepared from streptococci derived from human sources. The effects said to be produced in cases of chronic rheumatism are very remarkable, as, according to Menzer's account,<sup>3</sup> the injection of the serum gives rise to a reaction of an inflammatory nature at the seats of rheumatic lesions (the joints), which is followed by improvement. This peculiar result of the serum would suggest, as was pointed out by Blumenthal, that the serum is not antitoxic or antibacterial, but contains a toxin similar to tuberculin or mallein. The reaction is exactly parallel to that which follows an injection of the old tuberculin in a patient suffering from a tuberculous joint. Menzer, however, holds that his serum is antibacterial, and from what is known of its mode

<sup>1</sup> *Journ. of the American Med. Assoc.*, Jan. 3, 1903.

<sup>2</sup> *Gaz. des Hôp.*, April 4, 1903.

<sup>3</sup> *Zeitschr. f. klin. Med.*, 1902, xlvii. 109.

of preparation it should be of this nature. He suggests the explanation that the reaction is due to destruction of the cocci present in the patient and to the resulting rapid absorption of intracellular toxins. Symptoms of constitutional disturbance also accompany the injections of serum in rheumatic patients, viz. rise of temperature, headache, sore-throat, and enlargement of lymphatic glands. Cardiac disease, if present, may at first be aggravated by the remedy, but is subsequently improved. The local inflammatory symptoms are not seen in cases of gonorrhœal rheumatism which are treated with the serum.

Menzer gives in his original paper an account of seven cases of rheumatism treated by his serum, and holds that they were all improved. Relapse did not follow the treatment. In one case, which exhibited symptoms of nephritis, these were at first aggravated by the use of serum, but finally improvement was effected; indeed, Menzer suggests that the serum may prove useful in the treatment of some cases of chronic renal disease in which a local stimulating effect may be beneficial. He does not claim that an actual cure of rheumatic fever, or even a cutting-short of the disease, is directly effected by the serum, but thinks that by its means the resistance of the patient is raised. This theory would be consistent with an action analogous to that of tuberculin; which, however, Menzer will not allow.

A patient who had suffered from chronic rheumatism, and had been ineffectually treated by ordinary means, was shown by Menzer at the Berlin Medical Society.<sup>1</sup> As the result of serum treatment the man had so far improved that he could get about with sticks after four weeks' treatment.

The dose of the serum is not well established. Five to 10 c.c. may be given experimentally, but large quantities may be used if no ill effects are observed. Simnhuber<sup>2</sup>

<sup>1</sup> *Berlin. Verein f. inn. Med.*, March 23, 1903. See *Centralbl. f. inn. Med.*, 1903, p. 410.

<sup>2</sup> Quoted by Menzer, *Münch. med. Woch.*, 1904, p. 461.

supports Menzer in recommending the use of the serum in cases which have become chronic.

Very great interest attaches to Menzer's experiments, but at present no definite opinion can be expressed as to the merits of the treatment. Cases of acute rheumatism generally yield readily to the use of salicylates, and in view of this fact it is natural to hesitate somewhat before prescribing a remedy which seems capable of giving rise to alarming symptoms. It would seem, for the present at least, wise to refrain from using the new remedy in cases complicated by recent endocarditis or other acute lesions of important parts (pleurisy, pericarditis, etc.). In chronic cases, however, it might be safely tried. These are so rebellious to treatment by ordinary means that any method which holds out a prospect of success is to be welcomed. No danger seems to reside in the use of the serum in such instances, while it is easy to suppose that the local reaction might have a curative influence by its stimulating effect on indolent tissues.

**Chorea.**—The connection between this disease and rheumatism is not exactly ascertained. Many writers consider that chorea is a nervous manifestation of the rheumatic poison, while others see in rheumatism only one of several debilitating diseases which may cause the peculiar condition of the motor centres characteristic of chorea. Bacteriological examination has in some instances shown the existence in chorea of organisms similar to those which have been found in acute rheumatism, and some experimenters (Poynton and Paine, Walker) have produced in animals, by injection of these organisms, a condition which they consider to represent the chorea of human beings.

Preobrajensky<sup>1</sup> treated several cases of severe chorea, which he considered to be of an infective type, with a polyvalent antistreptococcic serum. Great improvement ensued, very rapid diminution in the symptoms being observed.

<sup>1</sup> Quoted in *La Semaine Méd.*, 1902, p. 412.

Relapses occurred in some of the cases, but renewed use of the serum caused their disappearance.

Chorea, like rheumatism, is a disease in which it is very difficult to make sure of the effects attributable to drugs. Much conflict of opinion has, for instance, been exhibited as to the efficacy of arsenic in this ailment; and other remedies have been vaunted as specifics, only to be rejected in the light of further observation. Hence the claims of antistreptococcic serum to cut short the disease must be closely scrutinized before they are accepted.

### 3. SCARLATINA

**Etiology.**—The causation of scarlatina or scarlet fever is not definitely established. Many observers have described cocci in connection with this disease, and there is a tendency to regard these as the exciting cause of the malady, but so far exact proof is wanting.

Mallory<sup>1</sup> has described "protozoon-like bodies" in the epithelial cells of patients who had died of scarlatina, and is inclined to consider them to be the causal agents. He named the organism (?) provisionally *Cyclaster scarlatinalis*.

In view of the great infectivity of scarlet fever, the contagion being conveyed from one person to another without direct contact, it seems improbable that the *Streptococcus pyogenes* can be the cause of the disease. Conditions due to infection with virulent forms of this organism seem to be propagated only by direct transference (hands, instruments, etc.), and it is unlikely that an attenuated germ, such as that of scarlatina would almost undoubtedly be, could be more readily communicated than the virulent kind. Provisionally, we may conclude that the disease is due to some organism at present unidentified, either owing to its ultra-microscopical size, or to failure to cultivate it outside the body. The constant association of streptococci with scarlatina would indicate that the

<sup>1</sup> *Journ. of Med. Research*, Jan., 1904, p. 483.

diseased tissues afford a favourable pabulum for these cocci, which take advantage of the opportunity thus offered, and are probably responsible for many of the complications of the malady, such as ulceration of the throat and disease of the middle ear, just as they appear to excite suppurative lesions in other acute diseases.

### SERUM TREATMENT

Moser,<sup>1</sup> who found that of 99 cases of scarlatina 73 exhibited cocci in their blood, prepared, by injecting horses with these organisms, a specific antiscarlatinal serum. This was tried by Escherich, apparently with good results. He records that the mortality was reduced by its means to 8.9 per cent., whereas in other hospitals at the same time, where the serum was not used, the death-rate amounted to 13.9 per cent. The injection of the serum was followed by fall of temperature and general improvement.

Bokay,<sup>2</sup> Zuppinger,<sup>3</sup> and Winocouroff<sup>4</sup> confirm these observations. Mendelsohn,<sup>5</sup> on the other hand, reports unfavourably, finding the serum quite useless.

Marpmann<sup>6</sup> prepared a "specific" serum by injecting animals with the blood of scarlet-fever patients, with emulsion of their epidermic scales collected in the peeling stage, and with their urine. He prepares a stronger serum for treatment and a weaker for prophylaxis. Campe<sup>7</sup> reports favourably on the value of the serum, which is called "scarlatin."

**Antistreptococcic serum.**—Baginsky<sup>8</sup> tried Marmo-

<sup>1</sup> Quoted in *La Semaine Méd.*, 1902, Appendices, p. clviii.

<sup>2</sup> *Jahrb. f. Kinderheilk.*, xii. 428.

<sup>3</sup> *Wien. klin. Woch.*, 1905, No. 44.

<sup>4</sup> *Ibid.*, p. 695. See also Egis and Langovoy, *Jahrb. f. Kinderheilk.*, 1907, xvi. 514; Eguez, *Rousski Vrach*, 1904, p. 1635; Schick, *Deut. med. Woch.*, 1905, p. 2092.

<sup>5</sup> *Ibid.*, 1905, p. 461.

<sup>6</sup> Abstr. in *Centralbl. f. inn. Med.*, 1905, p. 999.

<sup>7</sup> *Berlin. klin. Woch.*, 1905, No. 52.

<sup>8</sup> *Ibid.*, 1902, p. 394.

rek's serum in cases of scarlatina, but found that it did not produce any good effect on the course of the disease. Subsequently he made use of a serum prepared by Aronson, with better results, the mortality from the disease falling from 14 to 11 per cent. The figures do not seem very striking. Cumston<sup>1</sup> found a polyvalent antistreptococcic serum useful in "septic" cases of scarlet fever.

In view of the suppurative lesions which may occur as complications of scarlatina (ulceration of the throat, otitis media, etc.), it would seem advisable to use an antistreptococcic serum in severe cases, with a view to prevent or relieve these lesions. It can hardly be expected that the course of the disease itself will be modified by this procedure, but in this country scarlet fever is a mild disease, and it is rather the complications than the primary infection which are to be feared. In the anginose form the use of the serum would seem specially indicated, as this is almost certainly due to secondary infection. The mortality in these cases is high, and even in those which recover the convalescence is long and tedious. Mackie<sup>2</sup> has used antistreptococcic serum in these cases with good results.

**Serum of convalescents.**—Leyden<sup>3</sup> has observed the effects of injecting serum derived from convalescents from scarlet fever into patients suffering from the disease. He thinks that good results ensue. According to his observations, the disease is cut short, the temperature reaching normal some days earlier than it would otherwise have done. No critical fall of the fever is, however, seen. The doses administered were from 10 to 20 c.c., and no ill effects were produced.

These experiments are of theoretical rather than practical interest, as it is not to be expected that such a remedy could become generally used. Convalescents from a disease could not be expected to sacrifice a portion of their blood

<sup>1</sup> *Brit. Med. Journ.*, 1908, i. 1290.

<sup>2</sup> *Lancet*, 1904, i. 493.

<sup>3</sup> *Deut. Arch. f. klin. Med.*, Bd. lxxiii.



for the benefit of other patients--at all events, in this country. A perusal of Leyden's paper does not convey the impression that the observed results were at all strikingly encouraging.

#### VACCINE TREATMENT

Gabritschewsky<sup>1</sup> has prepared a vaccine consisting of a bouillon culture of streptococci isolated from cases of scarlatina, killed by heating to 60° C., and preserved by the addition of 0.5 per cent. of phenol. It is used as a prophylactic, 0.1 c.c. being given for each year of the child's age, with a maximum dose of 1 c.c. The vaccine has been extensively used in Russia, where severe epidemics of scarlet fever occur. Statistics seem to favour its employment in these circumstances. Thus Nikitin found the incidence of the disease 1.4 per cent. in vaccinated villages, 16 per cent. in those unvaccinated; and other observers give similar figures.<sup>2</sup>

<sup>1</sup> *Bull. Inst. Pasteur*, v. 575; *Centralbl. f. Bakt.*, I. Orig., 1906, xli. 377, 719. Cf. Langovoy, *ibid.*, 1906, xlii. (Orig.), 362, 463.

<sup>2</sup> See R. M. Smith, *Boston Med. and Surg. Journ.*, 1910, clxii. 242.

## CHAPTER XIX

### OTHER INFECTIONS DUE TO COCCI

#### PNEUMOCOCCIC INFECTIONS

**Causation.**—The organism to which the vast majority of all cases of acute pneumonia are due is the *Diplococcus pneumoniae*, or pneumococcus of Fränkel and Weichselbaum.

Another organism occasionally associated with pneumonia is the pneumobacillus of Friedländer (1883). This bacillus may also give rise to a form of membranous sore-throat, and has been found in abscesses in various parts of the body, as well as in pleurisy, endocarditis, otitis, rhinitis, etc. The pneumobacillus is *agglutinated* by the serum of patients infected by it, and exhibits chain-formation if grown in immune serum. A special phenomenon, “amorphous agglutination,” the nature of which is doubtful, is described by Schmidt.<sup>1</sup>

**Agglutination.**—The diplococci are agglutinated by the serum of immunized animals or of patients suffering from the disease. The reaction is not so easily demonstrated as in the case of *B. typhosus*, *Micrococcus melitensis*, etc.; and it does not appear to be of much practical value for clinical diagnosis. The phenomenon can be best shown by growing the cocci in some of the immune serum, a control growth being made in normal serum. In the latter the organisms produce a turbidity of the fluid, and under the microscope are seen to be uniformly scattered about in pairs or short chains. In agglutinative serum the organisms grow in flocculi, while the rest of the fluid remains clear.

<sup>1</sup> *Münch. med. Woch.*, 1903, No. 30, p. 873.

Microscopically the cocci are seen to be adherent in long chains or in clumps.<sup>1</sup> Jehle,<sup>2</sup> who apparently used the ordinary technique for agglutination experiments, reports that in children the reaction appears early in the disease, so as to be of diagnostic value, and that it may be useful for prognosis, since pure pneumococcic affections generally tend to recovery.

**Complement - fixation.** — Isabolinsky and Dichno,<sup>3</sup> using an antigen consisting of an extract of pneumonic lung, found a positive reaction in 9 out of 12 cases of pneumonia. Of the 3 which failed to give the reaction, 1 patient died and the other 2 were tested in a very early stage of the disease. Cases of pleurisy and influenzal pneumonia gave a negative reaction.

**Serum treatment.**—G. and F. Klempner<sup>4</sup> were the first to attempt to treat cases of pneumonia by means of an antagonistic serum. They prepared this from rabbits immunized by injections of pneumococci, using a precipitate from the blood of these animals which they called "antipneumotoxin." In 6 cases so treated these authors claimed good results. Washbourn<sup>5</sup> immunized a pony, and Parr<sup>6</sup> an ass; the latter treated 22 cases with the serum, with 2 deaths, both in patients who were moribund at the time of admission; he found that doses of 50 c.c. were followed by crisis and recovery. Biggs<sup>7</sup> immunized a horse, and found that the serum would protect rabbits against 1,000 lethal doses of the cocci; but he obtained only indecisive results in man.

Pane<sup>8</sup> prepared a serum from donkeys, and recorded

<sup>1</sup> See Eyre and Washbourn, *Journ. of Pathol.*, 1898, v. 13; Besançon and Griffon, *Ann. de l'Inst. Pasteur*, 1900, xiv. 449.

<sup>2</sup> *Wien. klin. Woch.*, Aug. 6, 1903, No. 32, p. 917.

<sup>3</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1912, p. 217.

<sup>4</sup> *Berl. klin. Woch.*, 1891.

<sup>5</sup> *Brit. Med. Journ.*, 1897, i.

<sup>6</sup> Quoted by Biggs, *infra*.

<sup>7</sup> *Med. News*, 1899, lxxv. 97, 137.

<sup>8</sup> *Riforma Medica*, 1898.

very favourable results. This serum was capable of protecting laboratory animals against 3,000 lethal doses. Eyre and Washbourn<sup>1</sup> found it effective against four out of five strains of pneumococci. Maragliano,<sup>2</sup> in 1898, stated that "he was more and more convinced of the antitoxic (?) power of Pane's serum, and of its efficacy in pneumonia." Fanoni<sup>3</sup> is very confident of the value of this preparation. He employs doses of 40 c.c. daily, and finds that the temperature is lowered, the general condition of the patient improved, and resolution of the affected lung accelerated. In children especially good results are to be obtained.

More recently Oreste<sup>4</sup> has written in favour of Pane's serum; but Horder and Scofield<sup>5</sup> found it useless in a case of pneumococcic *endocarditis*.

Lambert<sup>6</sup> immunized horses and treated cases with the serum. He found that slight reduction of the temperature was effected, but crisis was not induced. The pneumococci may disappear from the blood, if they are present there; in other words, a pneumococcic septicæmia may be prevented. The total effect obtained is, however, small; and Lambert has given up the use of the remedy.

Römer prepared a serum by injecting horses, cattle, and goats with different strains of pneumococci and mixing the serums thence obtained. It is favourably reported on by Knauth,<sup>7</sup> Paessler,<sup>8</sup> Tauber,<sup>9</sup> and others, doses of 10 to 30 c.c. being given. Dorendorf<sup>10</sup> states that Römer's serum is useless in cases of pneumonia, and this is our own experience.

<sup>1</sup> *Brit. Med. Journ.*, 1899, ii. 585.

<sup>2</sup> Quoted by Fanoni, *Med. Record*, March 10, 1900, p. 431.

<sup>3</sup> *Pediatrics*, May 15, 1900.

<sup>4</sup> *Gaz. degli Ospedali*, 1906, No. 22.

<sup>5</sup> *Lancet*, 1905, i. 1333.

<sup>6</sup> *Journ. of Amer. Med. Assoc.*, 1900, i. 901.

<sup>7</sup> *Deut. med. Woch.*, 1905, No. 12.

<sup>8</sup> *Deut. Arch. f. klin. Med.*, Hft. 3 and 4.

<sup>9</sup> *Wien. klin. Woch.*, 1906, No. 11.

<sup>10</sup> *Med. Klinik*, 1912, p. 1579.

Neufeld and Haendel<sup>1</sup> find that there are several strains of pneumococcus, so that a polyvalent serum is necessary. They prepared one which was curative for guineapigs infected with pneumococci. For use on man large doses must be given intravenously. Geronne<sup>2</sup> reports favourably on the remedy, finding that it brings down the patient's temperature and induces a feeling of well-being. It does not, however, lead to disappearance of the physical signs of disease in the lungs; indeed, he suggests that it may even delay resolution—which seems a doubtful recommendation.

With regard to the properties of a serum prepared by injections of *Diplococcus pneumoniae* into animals, it would be natural to expect it to be bactericidal, not antitoxic. On the other hand, it is found that the cocci will grow in the serum of patients suffering from the disease, and in that of immunized animals (*see* p. 373). Bokenham<sup>3</sup> states that the serum has no effect on the cocci by itself: if, however, it is brought into contact with the organisms in the presence of leucocytes, it causes these cells to destroy the germs by phagocytosis, i.e. it has an opsonic influence.

The value of serum in cases of *ulcus serpens corneae*, which is often due to a pneumococcic infection, is in dispute.<sup>4</sup> Allen<sup>5</sup> isolated a special coccus from cases of ulcerative keratitis and found vaccine treatment with it useful. The employment of a pneumococcus vaccine was ineffective.

**Serum of convalescents.**—Weisbecker<sup>6</sup> first employed this method of treatment in a series of 21 cases,

<sup>1</sup> *Arch. a. d. Kaiserl. Gesundheitsamt*, 1910, xxxiv. 166.

<sup>2</sup> *Ibid.*, p. 1699.

<sup>3</sup> *Brit. Med. Journ.*, 1900, ii. 1080.

<sup>4</sup> *See* Römer, abstr. in *Biochem. Centralbl.*, 1903, p. 66, and Gatti, abstr. *ibid.*, p. 158.

<sup>5</sup> "Opsonic Method of Treatment," 1908, p. 224.

<sup>6</sup> *Münch. med. Woch.*, 1899.

but, in spite of some subjective improvement, he considered that the results were inconclusive. Marchoux<sup>1</sup> thought that he observed good effects with this method.

**Vaccine treatment.**—At first sight vaccine treatment seems scarcely indicated in acute pneumonia, in which there is a general intoxication and probably septicaemia. Nevertheless, the attempt to influence the disease by this means has been made, and it would seem that in some cases of lobar pneumonia the crisis is accelerated by some days when vaccine is employed. Craig<sup>2</sup> records 6 cases in aged persons, who usually succumb to the disease, in all of which recovery took place. He used doses of 20-50 millions of pneumococci, administering a stock vaccine first until an autogenous preparation could be made. In a later publication he insists that the vaccine must be given early, and records 47 cases with 7 deaths, and a series in private practice of 20 cases with 1 death.

Cohendy and Bertrand<sup>3</sup> used sensitized vaccine, made of many strains mixed, and believed that (?) of 7 cases 6 were benefited, 1 negative.

Shennan<sup>4</sup> says that he used vaccines in 29 cases, and has no doubt of their value; he gave doses of 30-40 millions. Lyons<sup>5</sup> reports 4 cases in which there was general improvement; the disease declined by lysis instead of by crisis. Raw,<sup>6</sup> having used vaccines in 207 cases, finds it difficult to estimate their value. In many cases there was improvement in the general condition, but there was no acceleration of crisis, and some cases were quite uninfluenced. He began with doses of 50 millions, increased to 100 millions or even 150 millions.

<sup>1</sup> *Ann. de l'Inst. Pasteur*, 1899, xiii. 193.

<sup>2</sup> *Med. Record*, 1910, lxxvii. 259.

<sup>3</sup> *Compt. Rend. Soc. Biol.*, 1913, lxxiv. 532.

<sup>4</sup> *Med. Record*, 1912, lxxx. 427.

<sup>5</sup> *Brit. Med. Journ.*, 1913, i. 992.

<sup>6</sup> *Lancet*, 1912, i. 646.

Wynn<sup>1</sup> advocates early administration and large (!) doses, 25-50 millions. Wright, Morgan, Colebrook and Dodgson<sup>2</sup> find small doses in treatment of no value (159 cases); a single large dose during the incubation period may diminish the severity of a subsequent attack. Prophylactic injections were of use in reducing both morbidity and fatality among a susceptible population (negroes). Raw<sup>3</sup> collected statistics of 207 cases, of which 173 recovered; Leary,<sup>4</sup> 83 cases, with 71 recoveries. Whorlow<sup>5</sup> mentions a case of chronic pneumonia (due to pneumococci), in which 16 doses of vaccine (2½-300 millions) were given, with great improvement. Pio and Durand<sup>6</sup> refer to a case with abscess of the lung and empyema, attributable to the pneumo-bacillus, much benefited by a corresponding vaccine.

Vaccine treatment also proves useful in cases of localized infection by the pneumococcus. Thus Magruder and Webb<sup>7</sup> gave injections of 14 and 20 millions of pneumococci in a case of pneumococcic *otitis media*, with benefit; and Lyon<sup>8</sup> successfully treated a case of *empyema* which did not heal up after evacuation of the pus, by inoculation of the organisms (100 and 200 millions).

Robinson<sup>9</sup> gave injections of from 11 to 23 millions in a case of pneumococcic *peritonitis* with good effect; and Eyre<sup>10</sup> records cases of pneumococcic *pyemia* and of *peritonitis*, due to the pneumococcus, successfully treated by autogenous pneumococcic vaccines, and is convinced of the value of vaccine treatment in pneumococcic infections.

<sup>1</sup> *Lancet*, 1914, i. 354.

<sup>2</sup> *Ibid.*, 1914, i. 87.

<sup>3</sup> *Ibid.*, 1912, i. 646.

<sup>4</sup> *Boston Med. and Surg. Journ.*, 1909, cxvi. 714.

<sup>5</sup> *Lancet*, 1913, ii. 811.

<sup>6</sup> *Lyon Médical*, 1914, cxii. 775.

<sup>7</sup> *Laryngoscope*, Nov., 1907.

<sup>8</sup> *Lancet*, 1905, i. 1718.

<sup>9</sup> *Brit. Med. Journ.*, 1909, i. 651.

<sup>10</sup> Erasmus Wilson Lectures, *Lancet*, 1908, i. 539.



Chapple<sup>1</sup> found vaccine in doses of 5 millions useful in cases of *vulvo-vaginitis* in children, due to the pneumococcus, which is almost as common a cause of this disease as the gonococcus.

The present writers recommend quite small doses, viz. 5, 10, and 25 millions, the vaccine being invariably prepared from the organism isolated from the patient. The effect of such a vaccine upon the temperature and general condition of cases of empyema doing badly after operation is often particularly striking. *Broncho-pneumonia* due to the pneumococcus is also amenable to treatment with pneumococcus vaccine, especially chronic cases in children.

**Diphtherial antitoxin.**—Talamon<sup>2</sup> has treated 50 cases of pneumonia with antitoxin, many of them being alcoholic patients, and some of them old persons. The death-rate was 14 per cent., whereas previously, in cases treated symptomatically, it had been 37 per cent. He finds that if the serum is administered before the fifth day of the disease, the mortality is only 4 per cent.; whereas, if it is not given till after the sixth day, the death-rate rises to 24 per cent. He gives doses of 20 c.c., repeated if necessary; in grave cases he injects 20 c.c. at once, and repeats the dose on the following morning and evening. Bessoni<sup>3</sup> also recommends this treatment, reporting 21 cases in which it was used, with a mortality of 4 per cent.; among 79 other patients not so treated, the mortality was over 16 per cent. Legros,<sup>4</sup> who made use of antitoxin in some cases of pneumonia, failed to obtain any benefit. The same criticism probably applies to the use of diphtherial antitoxin in pneumonia as in septic conditions (*see* p. 359).

O'Malley<sup>5</sup> believes that antitoxin is a very valuable

<sup>1</sup> *Lancet*, 1912, i. 1685.

<sup>2</sup> *La Semaine Méd.*, 1901, p. 69.

<sup>3</sup> *Ann. de Méd. et Chir. Infantiles*, Feb. 15, 1899.

<sup>4</sup> *La Semaine Méd.*, 1901, p. 158.

<sup>5</sup> *Amer. Med.*, Jan. 17, 1903.

therapeutic agent in cases of *broncho-pneumonia* in children, especially in the forms which complicate other infective diseases (measles, influenza, etc.).

**Chemotherapy.**—Morgenroth<sup>1</sup> endeavoured to apply Ehrlich's ideal *therapia magna sterilisans*, by the use of a parasiticide without action on the cells of the host, to infection with the pneumococcus, and carried out researches on the efficacy of the substance known as æthyl-hydrocuprein hydrochlorate ("optochin") as a remedy for such condition. In mice this drug has a curative effect in the presence of a pneumococcal septicæmia, and experiments by Wright<sup>2</sup> show that when mixed with human serum it kills pneumococci. In human patients suffering from pneumonia it does not seem, according to this last observer, to be of much value, while it is possibly capable of inducing blindness owing to its action on the optic tract. The clinical failure of the drug may be due to its inability to reach the cocci in the consolidated lung-tissue and exudate. Other observers, however (Vetlesen,<sup>3</sup> Lenne,<sup>4</sup> v. Baermann<sup>5</sup>), believe that it may prove a useful remedy; they did not experience any cases of permanent ocular injury.

**Summary.**—The results obtained up to the present with antipneumococcic serum are disappointing. It is certain that good effects are produced in animals, but these are in the direction rather of prophylaxis than of cure. In man, by the time that symptoms of pneumonia have developed, the cocci have gained so firm a footing and have increased to such numbers that only a very potent serum of a bactericidal nature could be expected to act efficiently. Those which are at present obtainable do not seem to come up to this standard. It is also possible that the immune bodies formed in the lower animals do not find suitable

<sup>1</sup> Morgenroth and Levy, *Berl. klin. Woch.*, 1911, Nos. 34, 44.

<sup>2</sup> "Drugs and Vaccines in Pneumonia." London, 1914.

<sup>3</sup> *Berl. klin. Woch.*, 1913, No. 32.

<sup>4</sup> *Ibid.*, No. 43.

<sup>5</sup> *Zeitschr. f. exper. Pathol. u. Therap.*, 1914.

complements in man. Further, it appears that there are different strains of the pneumococcus which react differently to antibodies. More might be expected from an antitoxic than from a germicidal serum, as pneumonia is characterized by symptoms of profound intoxication; but as it has not been possible to obtain potent solutions of the toxins of the *Diplococcus pneumoniae*, no such serum is at present available, nor is there any immediate prospect of its preparation. Vaccine treatment of acute lobar pneumonia must still be regarded as in the experimental stage, but it already gives promise of future success; whilst vaccines in the treatment of chronic pneumonic affections are of considerable value. Chemotherapy of pneumonia is not at present to be very seriously regarded.

#### STAPHYLOCOCCIC AFFECTIONS

Staphylococci are probably always present on the skin of mankind. They are capable of giving rise to suppuration in favourable circumstances. Several varieties are generally described, *Staphylococcus pyogenes aureus*, *citreus*, and *albus* being the commonest. A general septicæmia due to any variety of staphylococcus may be produced in rare cases.

**Toxins of staphylococci.**—Denys found that by growing the staphylococci in fluid derived from serous exudates he could obtain a toxic substance which had the property of dissolving the leucocytes of the blood. The leucocytes, when treated with this substance, to which he gave the name of “leucocidin,” first became transparent, with clearly defined nuclei, and finally were broken up and destroyed. The importance of this toxin in the parasitic life of the bacteria is evident, since it is largely by means of the leucocytes that the infected individual resists the invading organisms. It is in this sense that we must look upon the formation of local collections of leucocytes (abscesses) at the points where the cocci settle. Leucocidin prepared from

cultures in rabbits does not appear to have the power of dissolving human leucocytes. Other poisonous substances which have the property of exciting suppuration have been obtained from the bodies of staphylococci. To one of these Leber gave the name of "phlogosin."

**Serum treatment.**—Several attempts have been made to obtain an antistaphylococcic serum. Antibodies can be obtained to the various poisons formed by the cocci, and rabbits can be immunized against the organisms. A serum capable of protecting these animals against several times the minimal lethal dose of the cocci has been prepared. It is doubtful if any good effects are to be looked for in man. The fact that the leucocidin which is active for rabbits' cells does not affect human leucocytes suggests that both the toxins and their antibodies may be different in different surroundings, so that animals' serum would be unlikely to have a curative action in human disease. Vicquerart and Doyen have prepared antistaphylococcic serums for purposes of treatment, but their value is doubtful. Moritz<sup>1</sup> tried antistaphylococcic serum in six cases of acute endocarditis (5 c.c. doses), and was favourably impressed by the apparent results.

Recently, Thomas<sup>2</sup> prepared a polyvalent antistaphylococcic serum by injecting a ram with a mixture of cultures, using first killed and finally living organisms. He gave doses of 15 minims (in infants) up to 4 c.c., and noted good results in cases of boils and carbuncles.

**Agglutination.**—Staphylococci are agglutinated by immune serum, and this reaction is suggested by Bruck, Michaelis and Schultze<sup>3</sup> as a means of diagnosis.

**Vaccination.**—Wright<sup>4</sup> inaugurated the use of dead cultures of the organisms as a vaccine in cases of acne and sycosis, with benefit.

<sup>1</sup> *St. Petersburg. med. Woch.*, 1898, No. 19.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, 1913, lx. 1070.

<sup>3</sup> *Zeitschr. f. Hyg.*, 1905, l. 144.

<sup>4</sup> *Lancet*, 1902, i. 874; *Brit. Med. Journ.*, 1904, i. 1075.

Stock vaccines are exceedingly useful in staphylococcic infections, though distinctly inferior in efficiency to auto-genous vaccines. Least useful of all are "mixed" vaccines of *Staphylococcus albus* and *aureus*. In every case the particular type of staphylococcus responsible for the lesion, whether aureus or albus, must be determined, and a corresponding vaccine be employed. Furunculosis, solitary boils and carbuncles, sycosis, periostitis and osteomyelitis, staphylococcic septicæmia and pyæmia are usually due to *Staphylococcus aureus*; acne indurata to *Staphylococcus albus*. Generally speaking, the control afforded by the opsonic index can be dispensed with, and the injections given in accordance with the clinical phenomena. For example, in a case of furunculosis the immediate result of an injection of vaccine is the appearance of a fresh crop of boils; the majority of these, however, quickly abort, and the condition improves. The appearance of another set of boils is the signal for a further injection of vaccine. The size of the dose is a matter for careful consideration, and can usually be gauged from clinical observations following the initial tentative dose. In treating boils, etc., if the infecting organism is diagnosed in the early stages, a dose of 10, 25 or 50 millions may cause rapid resolution. If, on the other hand, a chronic indurated focus of long duration is present, it is often advisable to administer a large dose of 250 or 500 millions, in order temporarily to depress the patient's resistance, and at the same time to hasten the breaking-down of the abscess by fomentations, etc., as a preliminary to evacuating the pus.

An average initial dose would be 50 millions in a localized suppuration, and a dose one-fifth of that in a generalized infection. When stock vaccines are employed, doses from twice to four times as large should be administered to ensure comparable results.

And here we would again insist on the principle that general and local treatment should be energetically carried out during the course of vaccine-therapy.

In some chronic conditions, e.g. furunculosis, which improve to a certain point and then appear to become stationary, it may be desirable to discontinue the vaccines for a time and then resume their use. On the other hand, it is in these cases that sensitized autogenous vaccines, at first of dead cocci and afterwards of living organisms, find their most strikingly successful application.

Gilchrist<sup>1</sup> has cured bullous erythema with vaccine. He states that affections due to *Staphylococcus aureus* may be cured by the use of an inoculation of *Staphylococcus albus*.

### PYORRHŒA ALVEOLARIS

Pyorrhœa alveolaris, or suppurative periodontitis (Rigg's disease), an acute or chronic inflammation of the gum-margins and periodontal membrane, often associated with arthritis and other distant lesions, and sometimes forming the starting-point for a general septicæmia, may apparently be the result of the pyogenic activities of any of the pathogenic bacteria, and, like suppurative processes elsewhere, can be influenced by means of autogenous vaccines. In this disease particular care must be exercised in determining the responsible micro-organism—at times by no means an easy matter—and frequently the infection is a mixed one. *Streptococcus pyogenes*, pneumococcus, *Micrococcus catarrhalis*, *M. tetragenus*, *M. paratetragenus*, *Staphylococcus aureus*, *B. pneumoniae*, and *B. coli* are among those most commonly found singly or in combination. Small doses, 5 or 10 millions, of the appropriate vaccine, injected every six to eight days, and gradually increased to 50 or 100 millions, give the best results. Treatment may need to be prolonged for three or even for six months, and should be associated throughout with careful local treatment by the dental surgeon.

Goadby<sup>2</sup> used a vaccine of *Staphylococcus aureus* suc-

<sup>1</sup> *Lancet*, 1908, ii. 471.

<sup>2</sup> *Brit. Med. Journ.*, 1908, ii. 477.

cessfully in 2 cases due to this organism; more recently<sup>1</sup> he recorded the presence of a streptococcus, which he appeared to regard as a streptobacillus, in the large majority of his cases in which arthritis was an associated symptom; and summarized the results from treatment with autogenous vaccine in 45 cases as 23 cured, 14 relieved, 4 failures, and the remainder under treatment.

Eyre and Payne<sup>2</sup> recorded 33 cases treated with autogenous vaccines (amongst these "rheumatism" was the outstanding clinical symptom in 18), of whom 20 were considered cured and 5 improved. The responsible organisms in these cases were *Streptococcus longus* (6), *M. catarrhalis* (6), a mixture of these two cocci (8), pneumococcus (3), and *Staphylococcus aureus* (2).

Medalia<sup>3</sup> finds vaccines specially useful in cases associated with staphylococci or pneumococci.

### EPIDEMIC CEREBRO-SPINAL MENINGITIS

Epidemic cerebro-spinal meningitis is characterized by a suppurative or fibrino-purulent leptomeningitis due to the *Meningococcus* or *Diplococcus intracellularis meningitidis*, discovered by Weichselbaum. The disease is very fatal apart from serum treatment, the mortality in New York being over 75 per cent.<sup>4</sup> (1906-8). Little is known of the toxins of this organism, which have not been obtained in culture-media.

**Diagnosis.**—The *agglutination* reaction occurs in most cases of cerebro-spinal meningitis, and may be used as a test for diagnostic purposes. It may, however, be absent during the first few days of the disease.<sup>5</sup> Young cultivations of a strain of meningococcus that has been

<sup>1</sup> *Lancet*, 1911, i. 639.

<sup>2</sup> *Proc. Roy. Soc. Med.*, Dec., 1909.

<sup>3</sup> *Boston Med. and Surg. Journ.*, Jan., 1910.

<sup>4</sup> Fischer, *N.Y. Med. Journ.*, Dec., 1909.

<sup>5</sup> Houston and Rankin, *Lancet*, 1907, i. 1213; 1908, ii. 474.



frequently subcultivated in the laboratory should be used for this reaction.

*Complement-fixation* tests and the estimation of the *opsonic index* have all in turn been advocated in the diagnosis of this condition, but all alike sink into insignificance beside the demonstration of the infective micro-organism in the cerebro-spinal fluid removed by lumbar puncture, more especially as the removal of the fluid forms such an important therapeutic measure in the treatment of the infection.

**Serum treatment.**—Serum for therapeutic purposes has been prepared by injecting animals with the cocci themselves, and is presumably bactericidal, not antitoxic.

Jochmann<sup>1</sup> obtained from horses, goats, and sheep a serum which agglutinated the cocci in dilutions of 1 : 300 to 1 : 15,000. He employed it on human patients, both subcutaneously and intrathecally, with good effects. Raczyński,<sup>2</sup> however, failed to observe any benefit from the use of this serum.

Flexner and Jobling<sup>3</sup> also prepared a serum which was used in 421 cases with a mortality of 33 per cent., or, excluding moribund cases, of 25 per cent. Robb<sup>4</sup> treated 90 cases with the serum, with a mortality of 30 per cent.

A serum prepared by Kolle and Wassermann has been used in Germany, Switzerland, and Italy. Többen<sup>5</sup> found that the mortality in his cases fell from 56·7 per cent. to 34·5 per cent.; and Krohne<sup>6</sup> similarly reduced the mortality from 66 to 47·6 per cent., the mortality in cases treated within the first two days being 33 per cent.

Currie and MacGregor<sup>7</sup> treated 105 cases with serum,

<sup>1</sup> *Deut. med. Woch.*, 1906, No. 20.

<sup>2</sup> *Wien. klin. Woch.*, 1907, No. 52.

<sup>3</sup> Studies from Rockefeller Inst., Reprints, 1909.

<sup>4</sup> *Lancet*, 1907, i. 1213.

<sup>5</sup> *Münch med. Woch.*, 1907, p. 2420.

<sup>6</sup> *Zeitschr. f. Medizinalbeamte*, 1908, No. 78.

<sup>7</sup> *Lancet*, 1908, ii. 1072.

using several different brands, with a mortality of 64·8 per cent., as compared with a mortality of 79·5 among 225 cases not so treated: the difference is not very striking. They collected from other sources the following statistical results in addition to those already mentioned: Wassermann, 57 cases, deaths 47·3 per cent.; Jochmann, 17 cases, deaths 29 per cent.; Robb (1908), 30 cases, deaths 26·6 per cent.; Dunn, 15 cases, deaths 20 per cent.; and Levy, 40 cases, deaths 11·76 per cent. The chief inference from the figures would appear to be that the disease varies much in severity at different times and in different places. Ker<sup>1</sup> treated 33 cases with Flexner and Jobling's serum, with a mortality of 40 per cent., and believes that the remedy shortens convalescence. Dopfer<sup>2</sup> records a total of 402 cases treated, with a mortality of 16·44 per cent., as against a mortality of 65 per cent. in the untreated. The doses used were 20–40 c.c. for adults and 10–20 c.c. for children, administered intrathecally.

During the end of 1914 and the early months of 1915 numerous cases of cerebro-spinal meningitis occurred amongst adults (chiefly in soldiers aggregated in training camps), the majority of whom were treated by the military surgeons with serum. The actual results are not yet available, but from personal communications as well as our own experience it would appear that polyvalent serums are essential and that the remedy must be injected intrathecally; given subcutaneously, serum is useless.

Mackenzie and Martin<sup>3</sup> used the *serum of convalescents*, with a mortality of 37·5 per cent.; and *diphtherial antitoxic* serum was tried by Huber,<sup>4</sup> Waitzfelder,<sup>5</sup> and Balduzzi,<sup>6</sup> with apparent benefit.

<sup>1</sup> *Edin. Med. Journ.*, Oct., 1908, p. 306.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1910, xxiv, 96

<sup>3</sup> *Lancet*, 1908, ii, 477.

<sup>4</sup> *New York Med. News*, April 16, 1905

<sup>5</sup> *Med. Record*, March 11, 1905.

<sup>6</sup> *Gaz. degli Ospedali*, 1907, No. 36.

Elder and Ievers<sup>1</sup> used injections of Rieni's *anti-pneumococcic serum* in cases of cerebro-spinal meningitis, on the ground of the relationship between the organism to which this affection is due and the pneumococcus, and thought that some improvement was produced. Cases of cerebro-spinal meningitis due to the pneumococcus or streptococci should be treated with the respective serums injected intrathecally in a similar manner, but in those due to the pneumococcus the prognosis is particularly grave.

**Method of injection.**—The serum must be injected intrathecally in amounts slightly less than those of the cerebro-spinal fluid withdrawn by lumbar puncture, probably 20–25 c.c. or 30 c.c. for adults and from 5 c.c. to 10 c.c. for infants. Sophian<sup>2</sup> strongly recommends the injection of the serum by gravity, regulating the amount of fluid withdrawn and the amount of serum subsequently injected by the movements of the blood-pressure. The withdrawal of fluid and the injection of serum must be repeated at intervals of twenty-four hours until the meningococci are found to have disappeared from the fluid withdrawn from the spinal canal.

**Vaccine treatment.**—Hector Mackenzie<sup>3</sup> treated a case with injections of a vaccine consisting of dead meningococci. The first dose consisted of 120,000,000 cocci, followed ten days later by 20,000,000, and again a week later by 143,000,000. A final injection of 71,000,000 organisms was given a week later. The vaccine was prepared from organisms grown from the patient's cerebro-spinal fluid obtained by lumbar puncture. The patient recovered. Rendle and Mottram<sup>4</sup> also made use of a vaccine of dead meningococci in doses of 200,000 and 500,000 organisms, and claim that benefit resulted from

<sup>1</sup> *Scottish Med. and Surg. Journ.*, 1907, xx, 215.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, 1912, p. 843.

<sup>3</sup> *Brit. Med. Journ.*, 1907, i, 1408.

<sup>4</sup> *Lancet*, 1907, ii, 220.

the treatment. We have treated three cases with vaccine but without averting the ultimate fatal issue.

The *opsonic index* of the blood is usually low (0·4) in cases of cerebro-spinal meningitis, and may be raised by injections of suitable vaccine, but, owing to the localization of the meningococci, vaccine treatment is not to be preferred to serum therapeutics. Probably the best results will be obtained by the combined use of antiserum and autogenous vaccine. The estimation of the opsonic index is occasionally of value as an aid to diagnosis.

Black<sup>1</sup> has carried out prophylactic vaccination against this disease, but no statistics are available as to its efficacy. He finds a positive complement-fixation reaction in the serum after such vaccination.

## GONORRHOEA

*Diplococcus gonorrhœe*, or the gonococcus, was first described by Neisser in 1879. It usually gives rise to a local suppurative affection (urethritis, conjunctivitis), but it may cause a general infection, the organisms entering the circulation and inducing arthritis, endocarditis, and septicæmia.

**Toxins and antitoxins.**—Christmas<sup>2</sup> grew the cocci artificially and obtained a poisonous fluid. A toxic solid, gonotoxin, can be precipitated from this by ammonium sulphate; it is not dialysable, and is not destroyed by heating to 65° C. for half an hour. By injection of the toxin into rabbits an antitoxic serum can be prepared, which neutralizes the poison *in vitro*, and acts prophylactically against injections of the poison in animals. The behaviour of vaccines sensitized by immune animal serums

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1913, lx. 289. In the recent epidemic outbreak of the disease in England many prophylactic inoculations have been carried out, the value of which it is impossible to estimate; and equally nebulous are the results of the vaccination of contacts, also extensively practised.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1900, xiv. 331.

seems to indicate that the antitoxic properties of such serums are inferior to their bactericidal powers.

### DIAGNOSIS

**Complement - fixation.**—Different conclusions have been reached by various investigators as to the value of the complement-fixation reaction as a test for the gonorrhœal infection. Much appears to depend on the nature of the antigen used. Thus, a solution of gonococci in antiformin was used by Merkurjew,<sup>1</sup> a suspension of the organisms in saline solution by Romanow,<sup>2</sup> and a similar emulsion by McDonagh and Klein.<sup>3</sup> The latter probably gives the more reliable results, but will not keep even at 0° C. for more than ten to fourteen days; the emulsion must not be concentrated, and an emulsion giving a count of 300 to 500 gonococci per cubic centimetre on titration will be found to form a suitable antigen.

Tcague and Torrey<sup>4</sup> observed that the serum of an animal immunized to one strain only of gonococcus might fail to fix complement in the presence of an antigen obtained from a different strain; hence they recommend an emulsion compounded from a number of different strains of gonococci as antigen.

McDonagh and Klein regard the test as a valuable aid to diagnosis, while Finkelstein and Gersham<sup>5</sup> find it useful in chronic cases, and Schwartz<sup>6</sup> in cases complicated by arthritis.

Lenartowecz<sup>7</sup> states that a positive reaction is obtained in 80–83 per cent. of cases of the disease. On the other

<sup>1</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1910, p. 864.

<sup>2</sup> *Ibid.*, 1913, p. 803.

<sup>3</sup> *Journ. of Path. and Bact.*, 1913, xvii. 559; *Proc. Roy. Soc. Med.*, Path. Sect., 1912, vi. 67.

<sup>4</sup> *Journ. Med. Research*, 1908, xvii. 223.

<sup>5</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1912, p. 243.

<sup>6</sup> *Amer. Journ. Med. Sci.*, 1912, cxliv. 369.

<sup>7</sup> *Dermatol. Woch.*, 1912, p. 1179.

hand, O'Neil<sup>1</sup> and Watabiki<sup>2</sup> look on the test as unreliable.

**Cutaneous reaction.**—A reaction analogous to the von Pirquet test in tuberculosis, obtained by inoculation of a glycerin extract of the cocci, is looked upon as useful by Irons,<sup>3</sup> but Seki,<sup>4</sup> Dmitrieff,<sup>5</sup> Sakaguchi and Watabiki<sup>6</sup> all hold that it is of no value. Attempts to constitute an ophthalmic test, analogous to the Calmette reaction for tubercle, also failed in the hands of Motomura.<sup>7</sup>

**Vaccine diagnosis.**—The administration of a large dose of gonococcus vaccine may be used for diagnostic purposes, being followed by a febrile reaction in 96 per cent. of cases and by a local reaction in 82 per cent. (Fronstein),<sup>8</sup> and by the reappearance of a positive complement-fixation reaction in the patient's serum. Gonococci previously absent from the urethral discharge may reappear as a consequence of this procedure. Some caution must be used in employing this test, as acute swelling of the uterine adnexa may ensue in women (Van der Velde).<sup>9</sup>

The authors find the most valuable application of this test in settling the question whether or not a patient who has suffered from gonorrhœa should be permitted to marry.

## TREATMENT

**Serum treatment.**—Rogers and Torrey<sup>10</sup> prepared a serum by injecting rams first with heated and then with fresh cultures of the gonococcus. They found it useless

<sup>1</sup> *Boston Med. and Surg. Journ.*, 1912, clxvii. 464; *Med. Record*, 1910, lxxviii. 599.

<sup>2</sup> *Journ. Infect. Dis.*, 1910, vii. 159.

<sup>3</sup> *Journ. Amer. Med. Assoc.*, 1912, lviii. 931.

<sup>4</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1910, p. 989.

<sup>5</sup> *Ibid.*, 1913, p. 253.

<sup>6</sup> *Dermatol. Woch.*, 1912, p. 717.

<sup>7</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1913, p. 772.

<sup>8</sup> *Ibid.*, 1913, p. 251.

<sup>9</sup> *Ibid.*, 1912, p. 538.

<sup>10</sup> *Journ. Amer. Med. Assoc.*, 1907, xlix., No. 11.

as a remedy in urethritis and conjunctivitis—localized lesions—but of some value in gonorrhœal septicæmia. Uhle and Mackinney<sup>1</sup> also record that serum treatment is useful in gonorrhœal arthritis, but ineffective against the urethritis caused by the gonococcus.

Bemskaja<sup>2</sup> prepared a serum by injecting a goat with gonococci, and found it of some use in treatment, although it appeared only to inhibit the development of the organisms rather than to kill them. The use of serum for gonorrhœal rheumatism is practised by L. E. Schmidt,<sup>3</sup> quantities of 2, 4, 6, 8 c.c. and so forth being administered every five days; and good results in this affection, as well as in orchitis, epididymitis, and cardiac complications, are recorded by Horwitz.<sup>4</sup> The authors regret that in their own experience they have never yet seen any beneficial effect follow the use of antigonococcic serums.

**Vaccine treatment.**—In cases of gonococcal urethritis of a subacute type the opsonic index is usually low (0·6); in those which rapidly convalesce it is high (1·8); whilst in those which merge into an intractable gleet it varies almost from day to day, within wide limits. Many writers regard vaccine treatment as inapplicable to the acute stages of the local disease, though Palmer<sup>5</sup> advises the use of small doses in this condition, and there is evidence that such treatment may relieve pain. On the other hand, there is a consensus of opinion as to the value of vaccines in localized complications such as epididymitis, orchitis, prostatitis, and iritis; whilst in severe cases of gonorrhœal sapræmia or septicæmia with pyrexia and prostration the effect of vaccines is most marked. Vaccination does not, according to Iljinsky,<sup>6</sup> pre-

<sup>1</sup> *Journ. Amer. Med. Assoc.*, July 11, 1908.

<sup>2</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1913, p. 945.

<sup>3</sup> *Med. Record*, 1910, lxxviii. 600.

<sup>4</sup> *Ibid.*, 1911, lxxx. 747.

<sup>5</sup> *Ibid.*, 1911, lxxix. 337.

<sup>6</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1914, p. 1074.



vent the occurrence of metastatic inflammation—a feature also noted in regard to tuberculin.

It is generally conceded that, in gonorrhœa, stock vaccines are as useful as autogenous, though O'Neil recommends the latter in cases of arthritis. A stock vaccine under the trade name of "Arthlignon" has enjoyed a certain vogue, also another termed "Dmego," stated to be a mixed vaccine of the gonococcus and a "syncoccus"—by which term is probably meant *Staphylococcus albus*, so frequently associated with the specific organisms in urethral discharges. In our hands the use of these proprietary vaccines in a long series of cases (with one notable exception which was rapidly cured apparently by Dmego) has been followed by distinctly unfavourable results. In any case the vaccines should not be kept for more than three months (Klausa),<sup>1</sup> as they undoubtedly deteriorate more rapidly in immunizing value than most bacterial vaccines.

Eyre and Stewart<sup>2</sup> advise that in the *acute stage*, whenever possible, autogenous vaccines should be employed; but if such a course is impossible, polyvalent stock vaccines, prepared from five or ten different strains of gonococci, must be used. Of these the initial doses should not exceed 5 millions. With such a dose the negative phase is, as a rule, short, and is marked clinically by exacerbation of the discharge, which lasts from one to three days, and is followed, as the positive phase becomes established, by a rapid diminution. After a second injection this sequence of events is repeated, but with a less marked negative phase. Urethral irrigation should at first be forbidden. Later, when the patient has shown a satisfactory response to the inoculations, irrigation of the urethra with some simple aseptic solution, such as potassium permanganate, 1:1,000, should be instituted, and it will usually be found that after a few days the discharge entirely ceases.

<sup>1</sup> *Berl. klin. Woch.*, 1913, xxxix. 1813.

<sup>2</sup> *Lancet*, 1909, ii. 76.

Sensitized vaccines (dead) prepared from autogenous or stock cultures of the gonococcus have proved very efficacious in our hands, but the sensitization must be effected either with human serum or with the serum of an animal immunized to that particular strain employed for the vaccine. McDonagh and Klein<sup>1</sup> have also laid stress on these points. These authors have besides reported favourably on the use of autolysed stock vaccines injected intravenously the dose—usually a small one of 1 to 5 millions—being distributed in some 10 c.c. of normal saline solution prior to administration.

One point of considerable importance must be insisted upon, namely, that cases of *chronic gleet* of many years' standing are frequently not benefited by gonococcus vaccine. The gonococcus in such cases appears to have died out, and various staphylococci and streptococci, often of very low virulence, are now responsible for the persistence of the "morning drop," and the administration of a vaccine prepared from such organisms rapidly results in a cure.

Eyre and Stewart<sup>2</sup> have summarized the vaccine treatment of gonococcus infections as follows :—

"1. *Acute gonorrhoea*.—1. Gonococcus vaccine is markedly toxic and exerts a profound influence over the disease.

"2. For routine work (hospital out-patients, etc.) vaccine treatment is not devoid of danger and requires the exercise of considerable caution.

"3. A stock vaccine, comprising a dozen different strains, gives results only slightly inferior to those observed when using a vaccine prepared from the patient's own organism. This is not the rule in most other diseases.

"4. Small doses, repeated at short intervals, are more effective than large doses at lengthened intervals.

"5. Small doses of vaccine (from 1,000,000 to 10,000,000 cocci) are safer and more satisfactory than the large doses (from 50,000,000 to 100,000,000) which are often prescribed.

"6. After an injection of from 500,000 to 2,000,000 the negative phase is either absent or extremely transient.

<sup>1</sup> *Journ. of Path. and Bact.*, 1912, xvii. 559.

<sup>2</sup> *Op. cit*

"7. An inoculation of from 5,000,000 to 10,000,000 causes a negative phase of usually not longer than forty-eight hours' duration, followed by a positive phase of from three to five days.

"8. Vaccine in *small* doses serves the double purpose of *raising and steadying* the opsonic index. A steady index just above normal is found to be the most favourable condition for rapid recovery.

"II. *Simple chronic gonorrhœa*.—1. Where the gonococcus has ceased to be the infecting organism, these cases are on a par with other chronic inflammatory states, but are frequently more difficult to cure owing to environment and local conditions.

"2. Chronic cases, where the gonococcus is the sole infecting organism, have a better prognosis from the point of view of treatment by vaccine than a mixed infection or one of staphylococcus only.

"III. *Chronic gonorrhœa with complications*.—1. The estimation of the opsonic index is helpful to diagnosis, and is a useful means of determining *approximately* the opsonic state of the blood. Chronic gonococcus infections, however, present clinical features which themselves afford valuable indications during the course of vaccine treatment.

"2. Where the gonococcus alone is the infecting organism, if the opsonic index cannot be obtained as frequently as is desirable, routine injections of from 1,000,000 to 2,000,000 cocci every three to five days are safe and satisfactory; a lapse of five to seven days after doses of 5,000,000; an interval of eight to ten days after inoculation of 10,000,000. Larger doses than these are seldom desirable.

"3. Treatment by small and gradually increasing doses at frequent intervals should at all times be preferred; the use of large doses is even more dangerous than in acute cases, and may be followed by disastrous consequences.

"4. In orchitis small doses of vaccine quickly relieve pain and cause a more rapid abatement of symptoms than is obtained by the usual routine treatment alone.

"5. In iritis the severe pain, which is a marked and obstinate feature, is relieved in forty-eight hours after an injection, and disappears in from three to four days; cure is much hastened.

"6. In arthritis the treatment is of considerable value."

## MEDITERRANEAN OR UNDULANT FEVER

**Etiology.**—This disease is due to a minute oval coccus, the *Micrococcus melitensis*, discovered by Bruce in 1888.

Paramelitensis fever is said to be due to a variant of *M. melitensis*, an organism which differs from *M. melitensis*, according to some observers, in its serum-reactions.

Working with various strains of *paramelitensis* supplied to us by Nicolle, we have been unable to satisfy ourselves of the existence of real differences.

**Agglutination.**—The micrococci are agglutinated by the serum of sufferers from the fever. The reaction may appear as early as the fourth day, seldom later than the sixth, and occurs in considerable dilutions (1 : 100, 1 : 1,000, up to 1 : 5,000). This reaction is very valuable as a means of diagnosis, but certain precautions must be observed in carrying it out. The culture of *M. melitensis* must be one recently isolated, or one recently passed through the body of an animal, and stable so far as concerns non-specific serum; the reaction must be positive in dilutions of 1 : 100 at least; and the frequent occurrence of inhibition zones or paradoxical reactions must be remembered, for it is quite common to observe a positive reaction in dilutions of 1 : 50 or 1 : 100 of a serum which fails to agglutinate the coccus in dilutions of 1 : 10 and 1 : 20.

The phenomenon of **complement-fixation** was obtained by Saisawa<sup>1</sup> with the serum of patients, the antigen used being an extract of the cocci. For practical purposes of diagnosis it is not likely to supersede the agglutination test.

**Serum treatment.**—Wright and Semple<sup>2</sup> have treated one case of this disease with a curative serum. The mode of preparation of this is not stated, nor can trial in a single case afford any trustworthy evidence of the value of the remedy.

Eyre<sup>3</sup> also prepared a serum from a horse, which agglutinated in dilutions of 1 : 3,000 to 1 : 5,000, and had some prophylactic power; but it did not prove very effective as a remedy for the disease.

A serum prepared in Italy, and known as Trambusti's or Trambusti and Donzello's serum, has been found useful

<sup>1</sup> *Zeitschr. f. Hyg.*, 1912, lxx. 177.

<sup>2</sup> *Lancet*, 1899, i. 1024.

<sup>3</sup> *Rept. Medit. Fever Commiss.*, 1907, Parts v., vi.

by Tomaselli,<sup>1</sup> Natale,<sup>2</sup> Cantieri,<sup>3</sup> and others, but the cases which they record are far from convincing.

**Vaccine treatment.**—Reid<sup>4</sup> used therapeutic inoculations, controlled by estimations of the opsonin-index, as a mode of treatment, with good results. Bassett-Smith<sup>5</sup> also prepared a vaccine by heating agar-cultures of the micrococcus to 60° C. for half an hour, but did not find it efficacious in the treatment of the disease.

Kennedy,<sup>6</sup> who states that agglutination is usually absent in chronic cases—pointing, perhaps, to lack of formation of other antibodies—finds autogenous vaccines useful, the fever tending to fall and the agglutinins to appear in the blood after this treatment. He gives an initial dose of 100 millions of cocci, followed by smaller doses of 6, 7½, or 9 millions.

We have obtained excellent results in a number of cases by the employment of autogenous vaccines in small doses, ½ to 1 million cocci, repeated at intervals of four to five days and gradually increased to 5 and 10 millions.

Eyre<sup>7</sup> employed similar vaccines in doses of 200, 300, or 400 millions for *prophylaxis*, and considered that some protection was thereby afforded.

<sup>1</sup> *Gaz. degli Ospedali*, 1912, xxxiii. 457.

<sup>2</sup> *Riv. Crit. Clin. Med.*, 1912, xiii. 787.

<sup>3</sup> *Ibid.*, 1915-16, pp. 289, 305, 321.

<sup>4</sup> *Ann. Rept. Sanit. Com. with the Govt. of India for 1905*, p. 153.

<sup>5</sup> *Journ. of Hyg.*, 1907, vii. 115.

<sup>6</sup> *Journ. Roy. Army Med. Corps*, 1910, xv. 317.

<sup>7</sup> *Rept. Medit. Fever Commiss.*, 1907, Part vi., p. 115.

## CHAPTER XX

### CATARRHAL AFFECTIONS

#### HAY-FEVER

**Etiology.**—Not only the peculiar specific affection connected with the pollen of certain grasses, but also nervous conditions of an asthmatic type, occurring in the summer months, are probably often included under the name of hay-fever. Dunbar<sup>1</sup> has satisfactorily proved that the pollen of rye and other grasses is responsible for true hay-fever. The pollen-grains contain a soluble toxin which is capable of affecting susceptible persons, whereas normally constituted individuals suffer no ill effects from it. Dunbar's experiments were as follows: He took the pollen of rye and applied it to the nostrils of a certain number of persons who suffered periodically from hay-fever, and also to those of others who had not experienced the disease. In the former definite symptoms of coryza and irritation of the nasal mucous membrane were produced; while the latter did not exhibit any ill effects. Similar results were produced in susceptible persons by applying the pollen to the conjunctivæ. That the symptoms were not due to the mechanical effects of the grains of pollen was proved by control experiments with materials derived from other plants, including those varieties which have pollen-grains of a prickly form. Of two persons who were placed in a room and caused to blow into a vessel containing the toxic pollen, one who was susceptible to hay-fever exhibited symptoms of bronchitis and asthma, while the other was unaffected.

<sup>1</sup> "Zur Ursache und Spezifischen Heilung des Heufiebers," 1903, and *Deut. med. Woch.*, 1903, No. 9.

The soluble nature of the toxin was demonstrated by making aqueous and ethereal extracts of the pollen and applying them in the same way to patients. Symptoms of hay-fever were invariably produced in susceptible persons. If the pollen is kept for any length of time, so that it becomes dry, it no longer induces symptoms; but if the dried grains are broken up, the toxic effects are again manifested. In ordinary cases it seems that the poison is dissolved out by the tears and nasal secretion, and is thus enabled to act. Dunbar found that symptoms of irritation were produced by application of the toxic pollen to other mucous surfaces besides those of the eye and nose, e.g. by application to the anus. If a solution of the poison is injected hypodermically, very similar effects are produced to those seen after its local application. Thus, a medical man, subject to attacks of hay-fever, received an injection hypodermically in the arm. He first felt giddy, and in a quarter of an hour was seized with sneezing, cough, lachrymation, soreness of the throat, and dyspnoea. The face swelled and the voice became hoarse; there was respiratory stridor, and by laryngoscopical examination the vocal cords were seen to be congested. The frequency of both pulse and respiration was increased. A cutaneous eruption ensued, of an urticarial nature, with great itching; the arm in which the injection was given became markedly swollen. A second subject, who did not suffer from hay-fever exhibited no ill effects from a similar injection.

It has been suggested that the phenomena of hay-fever are analogous to those of anaphylaxis.

**Antitoxin.**—Dunbar proceeded to attempt the manufacture of an antitoxin to counteract the toxin contained in the pollen. He injected the latter into rabbits, and used their serum for experiments. He found that the serum obtained from these animals was capable of neutralizing the toxin and protecting susceptible persons from its effects. Thus, if some of the serum were added to the toxin, the mixture could be introduced into the eye of a susceptible



person without any ill effects. Serum from a normal rabbit had no protective action.

Several other writers confirm Dunbar's results. Semon<sup>1</sup> tried the serum, and found that definite effects were produced. It cannot be described as curative of the disease, but considerable improvement results from its use. The effects differ considerably in different cases. If the serum be applied to the nose and eyes, when the first symptoms are experienced which are known by the patients to portend an attack of hay-fever, the threatened access may be aborted. In some cases the remedy unaccountably fails. The subjective relief produced in patients is often greater than the diminution in the objective signs of the disease. The duration of the relief afforded is not long, and repeated instillations of the antitoxin are required. Subcutaneous injections are not advisable, as the local œdema produced is considerable, and the amount of protection gained is uncertain.

M'Bride,<sup>2</sup> too, is favourably disposed towards the use of the serum. He concludes that Dunbar has definitely succeeded in isolating the toxin of the disease and in producing an antitoxin capable of neutralizing it. It is doubtful, however, whether it is the pollen of grasses alone which is responsible for the disease, and therefore whether we possess in the serum an antidote to all forms of hay-fever. Some persons suffer from a similar catarrhal condition if they are exposed to the smell of horses or cats, and it is evident that the serum prepared from pollen cannot be expected to act beneficially in such cases. Knight<sup>3</sup> collected 219 cases in which the serum was used; of these 114 show great improvement and 66 slighter benefit.

Thost<sup>4</sup> also confirms Dunbar's results, but remarks

<sup>1</sup> *Brit. Med. Journ.*, 1903, ii. 123, 220.

<sup>2</sup> *Edin. Med. Journ.*, 1903, ii. 7.

*Med. Record*, March 10, 1906.

<sup>4</sup> *Munch. med. Woch.*, June 9, 1903. Cf. Lübbert, *Therap. Monatsh.*, Dec., 1904.

that in all cases of hay-fever there is a certain element of nerve-weakness, which cannot be influenced by the serum; while the cases which are complicated by morbid local conditions will need appropriate treatment for these, as well as the specific remedy.

Weichardt<sup>1</sup> points out that in many cases the serum may do good at first, but is afterwards not tolerated. We have seen a certain number of instances in which it seemed to afford relief, but seldom, if ever, a result that could be described as a cure. Nevertheless the remedy is well worthy of trial in severe cases of the affection.

**Autumn catarrh.**—In the United States of America there is a form of hay-fever, known as “autumn catarrh,” which appears in the autumn, at a time when there is no pollen from rye in the air. It seems necessary to suppose that these cases are due to a different cause. Dunbar<sup>2</sup> has investigated some of the plants which might be responsible for such autumnal cases, and finds that golden-rod and rag-weed contain toxins capable of exciting the disease. The toxin is not the same body as that found in grass-pollen, but the antitoxin prepared for the latter is capable of neutralizing it to some extent. Dunbar considers that this action is comparable with the agglutinative action which the serum of a patient suffering from one disease may at times exert on bacteria other than the causal organism but belonging to the same group.

Dunbar adds some reasons for the want of success which at times attends the use of the serum as a cure for hay-fever. Patients often insist on sleeping with their windows open, and in otherwise exposing themselves to repeated infection with the toxin. They should, on the contrary, refrain from walks in the country at the times when they are liable to the disease, and generally avoid all opportunities of reinfection.

**Dunbar's serum.**—This is now prepared from horses,

<sup>1</sup> Abstr. in *Centralbl. f. Bakt.*, 1906, xxxviii. 493.

<sup>2</sup> *Berl. klin. Woch.*, July 13, 1903.

and is to be obtained commercially. It is called "pollantin," and is sent out in small cases containing a bottle of the antitoxin and a drop-pipette for applying it. The following directions<sup>1</sup> for its use are supplied:—

"As the serum would soon be contaminated by frequent contact with the pipette, it is advisable to pour out one-third of the contents of the serum phial into the empty glass provided with a drop-pipette. This glass is effectually closed by pressing the indiarubber cap of the pipette into it, but care should be taken to keep this glass upright, so as to avoid the liquid flowing into the cap. Patients should not fail to carry a small quantity of serum in this glass about with them whenever they may expect a hay-fever attack. Immediately after noticing the first symptoms of irritation in nose or eye, a drop or two of the serum should be instilled upon the eye or into the nose affected.

"The serum can be applied to the eye in the following manner: After sucking up a few drops into the pipette, exert a gentle pressure on the rubber cap until one drop just emerges from the opening of the pipette; then before a mirror carefully approach the pipette to the outer corner of the eyelid, when the drop is sucked up by the eyelashes, and spreads over the conjunctival membrane. By the aid of a pocket mirror the same manipulation can easily be carried out in the open air.

"In order to instil the serum into the nasal cavity, fill the pipette with three or four drops of serum, and, bending your head backwards, insert the pipette about  $\frac{1}{2}$  in. into the nostril affected, and empty it by a short pressure on the rubber cap. A few sniffs suffice to spread the serum over the mucous membrane."

**Ophthalmic test.**—Noon<sup>2</sup> devised an ophthalmic test for hay-fever on the lines of Calmette's ophthalmoreaction for tuberculin, by extracting 1 grm. of the pollen of *Phleum pratense* in 50 c.c. of water, to which he gives

<sup>1</sup> From Semon's article in the *Brit Med. Journ.*, 1903, ii. 124.

<sup>2</sup> *Lancet*, 1911, i. 1572.

an arbitrary value, stating that it contains 20,000 pollen units per c.c. From this varying dilutions are prepared in strengths of from 5 to 5,000 units. The healthy individual yields no reaction whatever, even when a few drops of the dilution of 5,000-unit strength are instilled into the conjunctival sac; conversely, most sufferers from true hay-fever will give a positive reaction (tickling sensation at inner canthus, reddening of the caruncle, or finally injection of the entire bulbar conjunctiva, lachrymation, and sneezing) even with the 5-unit dose.

**Vaccine treatment.**—Further experiments showed that immunization against the toxins of a number of different pollens could be effected by the subcutaneous inoculation of increasing doses of the pollen-extract or vaccine, the injections as to time and dose being controlled by means of the ophthalmo-reaction. Freeman<sup>1</sup> records satisfactory results in 16 patients out of 20 treated, and later<sup>2</sup> gives details of 64 additional cases; he is convinced of the value of the method. The pollen-vaccine may be used both as a remedy and as a prophylactic measure. Personally we prefer it in its latter aspect, and find that treatment commenced in February or March gives the most satisfactory results.

#### NASAL CATARRH

Allen<sup>3</sup> states that "a cold in the head" or coryza may be due either to Friedländer's bacillus, to the *Micrococcus catarrhalis*, or to *B. septus*. Vaccine treatment may cure chronic colds, and may prevent recurrence in persons who are prone to them. He uses a vaccine consisting of 150 millions of *M. catarrhalis* in cases due to this organism (which may also be responsible for tracheal catarrh). Our own experience suggests that it is advisable to prepare the vaccine from the organism derived from the individual

<sup>1</sup> *Lancet*, 1911, ii. 814.

<sup>2</sup> *Ibid.*, 1914, i. 1178.

<sup>3</sup> *Ibid.*, 1909, ii. 1589, 1659.

patient, and, when the cure is complete, to administer two or three doses of a stock vaccine compounded of the various bacteria which may be responsible for acute and chronic catarrh—viz. *Bacillus influenzae*, *B. septus*, *B. pneumoniae*, *Micrococcus catarrhalis*, and *M. pneumoniae*—as a prophylactic measure. We are convinced, too, that the dose above recommended is too large, and rarely give more than 25 (or 50 at the outside) millions during the course of treatment, or more than 100 millions for prophylaxis.

In many instances the duration of the immunity so produced is brief, and patients may need two or three prophylactic doses every autumn. On the other hand, individuals previously subject to chronic catarrh may acquire, as a result of vaccine treatment, freedom from this form of infection for a period extending to six years.

## CHAPTER XXI

### DISEASES DUE TO PROTOZOA

#### SYPHILIS

**Etiology.**—It is now generally accepted that the causal organism of syphilis is the spirochæte discovered by Schaudinn, and known as the *Treponema pallidum* (*Spirochaeta pallida*), a protozoon which has been grown in pure culture in artificial media by Noguchi.

**Serum reaction in syphilis.**—The appearance of the Bordet-Gengou phenomenon of complement-fixation (see Chapter IV., p. 89) in syphilitic infection was first brought forward as a practical means of diagnosis in the communication from Wassermann, Neisser, and Bruck,<sup>1</sup> in May, 1906. The work on which this was founded was done on Neisser's apes, artificially inoculated with syphilis. Immediately afterwards, and independently, Detre<sup>2</sup> tried the test on human patients, finding a positive reaction in 2 out of 6 cases. Further observations tending to substantiate the value of the method were afterwards published by Wassermann and Plaut.<sup>3</sup> Later, Morgenroth and Stertz<sup>4</sup> found positive reactions in the cerebro-spinal fluid of general paralytics, Schutze<sup>5</sup> in tabes dorsalis, and Bab<sup>6</sup> in the milk of syphilitic women.

The antigen originally used by Wassermann and his collaborators was a watery solution of the organs of

<sup>1</sup> *Deut. med. Woch.*, 1906, p. 745.

<sup>2</sup> *Wien. klin. Woch.*, 1906, ix. 619.

<sup>3</sup> *Berl. klin. Woch.*, 1907, Nos. 50 and 51.

<sup>4</sup> *Virchow's Archiv*, 1907, clxxxvii. 166.

<sup>5</sup> *Berl. klin. Woch.*, 1907, xlv. 126.

<sup>6</sup> *Centralbl. f. Bakt.*, I. Orig., 1909, li. 250.

syphilitic apes or of those of congenitally syphilitic children, or alternatively extracts of condylomata or syphilitic placentaë. Weygandt found that watery extracts of normal organs might act equally effectually as an antigen; while Landsteiner, Müller, and Potzl tried alcoholic extracts of syphilitic material, and Meier similar extracts of normal organs, with success. It is plain from these findings that the reaction is not a specific immune reaction between antigen, copula, and complement as in hæmolysis, since the antigen may be obtained from normal as well as from syphilitic tissues, and is not a specific product of the action of the *Spirochæta pallida* or a protein derived from the bodies of these organisms. Cholesterin and lecithin are each said to be capable of taking the place of the antigen, and it is generally supposed that some lipoid body is the substance normally at work. The exact nature of the reaction must at present remain in doubt.

According to Wassermann's technique the hæmolytic copula used is obtained by inoculating a rabbit intraperitoneally with washed sheep's corpuscles; the animal is then bled to death when sufficient injections have been administered, the blood defibrinated and centrifugalized, and the serum kept on ice. Before use it is heated for half an hour. Fresh guineapig's serum serves as the source of complement. Washed sheep's corpuscles are suspended in normal saline so as to form a 5 per cent. emulsion (by bulk). The antigen is obtained by mincing up syphilitic material, adding 4-5 c.c. of saline fluid (containing 0.4 per cent. of phenol) to each gramme of minced material, shaking for twenty-four hours, centrifugalizing, and decanting the clear fluid. The patient's serum is heated for half an hour to 56° C. before use. An antigen commonly used at the present time is obtained by mincing up normal heart-muscle (human or guineapig) and shaking with alcohol (10 c.c. to 1 grm.); the fluid extract is diluted with 9 parts of saline before use.

The technique of the procedure is as follows: The



serum from the patient, previously inactivated by heating (therefore containing only immune body), mixed with liver-extract and a small quantity of fresh serum from the guineapig (to provide complement), is incubated at 37° C. for one hour; to it are then added sensitized red cells (i.e. red cells mixed with their appropriate, but inactivated, hæmolytic serum), and the mixture is again incubated for an hour at 37° C., removed to an ice-chest, and allowed to stand for several hours before examination. To act as controls, a similar system is put up, in which the suspected serum is replaced by that from a normal individual, and another with serum from a known syphilitic. If syphilitic antibodies are present in the suspected serum, no hæmolysis will take place, as the complement required by the sensitized red cells for this reaction to occur have been already utilized and bound by the syphilitic immune body to the lipoid of the liver-extract. This constitutes a positive reaction. In the absence of immune body from the suspected serum, however, complete hæmolysis will take place and a negative reaction is recorded.

By varying the relative quantities of the reagents concerned in the "*syphilitic system*" the intensity of the Wassermann reaction is capable of measurement, and the test thus modified possesses a quantitative as well as a qualitative value—a point of importance as a guide to the progress of treatment.

To attain this end, some observers dilute the serum and test varying quantities of the dilution; others vary the amount of antigen; but the best method entails the use of varying units of complement, thus estimating the number of hæmolytic doses of complement fixed by any given serum in the presence of constant volumes of antigen and copula.

In reporting the results obtained, arbitrary signs are often employed: thus the + sign indicates a positive reaction as shown by total inhibition of hæmolysis. The - sign, of course, indicates a negative reaction, i.e. complete hæmolysis; whilst the combination  $\pm$  would indicate a

positive reaction in which a certain small amount of hæmolysis had taken place. Some observers record a strong positive reaction as + + + +, and indicate increasing degrees of hæmolysis by + + +, + +, +, and complete hæmolysis by 0.

Various modifications of the Wassermann procedure have been proposed. Thus, Porges and Meier<sup>1</sup> use lecithin as antigen; Bauer<sup>2</sup> makes use of the natural copula present in human blood instead of using artificial hæmolytic serum; Hecht<sup>3</sup> uses both the natural copula and also the complement of human blood, not heating the patient's serum; and Margarethe Stern uses the human complement with artificial copula.<sup>4</sup> The details of these modifications must be studied in the original papers.

**Wassermann reaction in syphilitic cases.**—Boas<sup>5</sup> states that in primary syphilis the test may be positive or negative; in any case the reaction is not positive in the cerebro-spinal fluid. In secondary syphilis the reaction is always positive with serum, if the patient has not been treated. Levaditi and Yamanouchi<sup>6</sup> found that the cerebro-spinal fluid only yields positive results when the nervous system is involved. In tertiary syphilis the reaction is always positive in the serum, but the cerebro-spinal fluid is usually negative. In tabes dorsalis the cerebro-spinal fluid always gives the reaction, and this is the case in 94 per cent. of cases of general paralysis. Marcus<sup>7</sup> gives the following figures: The percentage of positive reactions in cases of primary syphilis is 63, in secondary syphilis 98, in tertiary syphilis 100, in tabes dorsalis 60, in general paralysis 100.

<sup>1</sup> *Wien. klin. Woch.*, 1908, xxi. 206.

<sup>2</sup> *Deut. med. Woch.*, 1908, p. 698; *Semaine Méd.*, 1908, p. 429.

<sup>3</sup> *Wien. klin. Woch.*, 1908, p. 1742; 1909, p. 338.

<sup>4</sup> *Zeitschr. f. Immunitätsforsch.*, 1908, Orig. I., 432.

<sup>5</sup> "Die Wassermannsche Reaktion." Berlin, 1911.

<sup>6</sup> *Compt. Rend. Soc. Biol.*, 1907, lxii. 240; 1908, lxiv. 349.

<sup>7</sup> *Inaug. Diss.*, Stockholm, 1910. *Abstr. in Zeitschr. f. Immunitätsforsch.*, 1910, p. 1003.

Plaut<sup>1</sup> obtained a positive reaction in 80 per cent. of cases of undoubted syphilis.

Bassett Smith<sup>2</sup> finds the reaction positive forty-five days after infection; our own observations lead us to believe that the reaction becomes positive as soon as spirochaetes can be demonstrated in serum from the primary lesion.

**Wassermann reaction in other conditions.**—The tropical disease, *yaws* or *frambæsia*, is so closely allied to syphilis that many authorities have considered them to be identical. The causal spirochaetes are practically indistinguishable, and the symptoms are closely similar; only the failure of one condition to produce immunity to the other affords evidence of their diversity. It is therefore not remarkable that a positive Wassermann reaction should be met with in the subjects of yaws. Another spirochætal disease, *relapsing fever*, may exhibit the same phenomenon, as may also *ulcus tropicum* and *malaria*. The frequency of a positive reaction in cases of *leprosy* is disputed. The only important disease of temperate climates in which the reaction is often found positive is *scarlatina*. A positive test has also been found in patients who have inhaled anæsthetics (Wolfsohn),<sup>3</sup> in cases of lead-poisoning (Field),<sup>4</sup> and in one of sarcoma, in which last it became negative after the removal of the tumour (Lautenschläger).<sup>5</sup> We have ourselves obtained a positive reaction in a case of hæmo-sarcoma of the liver. It is thus apparent that no serious errors of diagnosis are likely to arise from positive reactions in conditions other than syphilis. Nevertheless the non-specific nature of the reaction is confirmed by these findings. The test is unreliable if applied to serum taken after death.

**Significance and variations of the reaction.**—Apart from the few diseases mentioned above, a definitely

<sup>1</sup> *Zentrabl. f. Nervenheilk.*, 1908, Hft. 8.

<sup>2</sup> *Brit. Med. Journ.*, 1910, ii. 1434.

<sup>3</sup> *Deut. Med. Woch.*, 1910, p. 505.

<sup>4</sup> *Journ. Amer. Med. Assoc.*, 1912, lvii. 1681.

<sup>5</sup> *Arch. f. Laryngol.*, 1912.

positive Wassermann reaction points to the presence of active syphilitic infection. A negative test is not conclusive against such a condition, but renders its existence improbable apart from convincing clinical symptoms. A positive reaction in a patient's cerebro-spinal fluid points to syphilitic disease of the central nervous system, either of the gummatous variety or of the late parasymphilitic manifestations, *tabes dorsalis* and *dementia paralytica*.

As a result of treatment with mercury the Wassermann reaction may become negative, but this change does not invariably occur. If after such disappearance the test again becomes positive, it is supposed that treatment has been inadequate and that the infection persists, rendering danger of relapse imminent. The effect of treatment with salvarsan and neo-salvarsan is also in many cases to cause disappearance of the Wassermann reaction, but here again the effect is not constant. Lange<sup>1</sup> gives the following statistics: Out of 268 cases, 153 which were at first positive became negative after treatment with salvarsan; of 18 which were negative, 13 remained so, but 5 became positive; while 93 cases remained positive throughout. It is certain that in cases which have been treated the negative issue of the test is of no importance as an indication that the disease was not originally syphilis. Fischer<sup>2</sup> holds that the reaction affords no clear indication either as to prognosis or treatment.

**Luetic reaction.**—Noguchi<sup>3</sup> has devised a diagnostic test for syphilis analogous to the intradermal tuberculin reaction. Cultivations of many different strains of *Spirochaeta pallida* are emulsified, sterilized by heating to 60° C., and preserved by the addition of 0.5 per cent. trikresol: 0.07 c.c. of this preparation, termed "lueticin," is injected into the substance of the skin—not hypodermically; the small wheal thus produced disappears in

<sup>1</sup> *Berl. klin. Woch.*, 1910, p. 1656.

<sup>2</sup> *Arch.f. Dermatol. u. Syph.*, 1910, c. 215.

<sup>3</sup> *Journ. Exper. Med.*, 1911, xiv. 557.

about ten minutes, and in the positive reaction is replaced in from twenty-four to seventy-two hours by a skin-lesion ranging from a small papule to a large necrotic ulcer.

**Serum therapeutics.**<sup>1</sup>—Since the lower animals, with the exception of the ape, are immune to syphilis (although corneal and testicular lesions can be produced in rabbits and guineapigs by the local inoculation of syphilitic material rich in spirochaetes), various attempts have been made to influence the course of the disease by injecting the serum of animals. Richet and Hericourt injected the serum of dogs; and other writers have recorded experiments with serums of other species (lambs, Tommasoli; horse, Kannberg; cattle, Kollmann). No satisfactory results have been obtained by this means.

Injection of syphilitic blood into animals, and inoculation with other products of the disease (hydrocele and ascitic fluid, chancres), have also been tried, the serum being then drawn off, and used therapeutically (Mazza, Gilbert and Fournier). Improvement is stated to have ensued in some of the patients thus treated. Risso and Cipollina<sup>2</sup> used a serum derived from dogs and asses, in doses of 2–5 c.c., and saw good effects produced in all stages of the malady.

Query,<sup>3</sup> who believes that the treponema is a stage in the development of a bacillus which he isolated, made use of this for immunizing animals, and thence prepared a serum, which Hallopeau<sup>4</sup> believed to be of value in treatment.

The serum of patients in the tertiary stage of the disease, and that of congenitally syphilitic infants, has also been employed. Good results are stated to have been attained in some cases by this means (Gilbert and Fournier, Boeck,

<sup>1</sup> For the literature on this subject, see Fouquet, *Gaz. des Hôp.*, Oct. 10, 1903, p. 1153; also Lane, *Practitioner*, July, 1904.

<sup>2</sup> *Rif. Med.*, Nov. 30, 1904; March 18, 1905.

<sup>3</sup> *Compt. Rend. Soc. Biol.*, March 9, 1907.

<sup>4</sup> *Ibid.*, Dec. 21, 1907.

Moore, etc.), but it is evident that such a mode of treatment could never be generally used.

The treatment of syphilis has now, however, for all practical purposes settled down to the alternate exhibition of two drugs, salvarsan and mercury.

**Chemotherapy.** — The term chemotherapy, which, rightly speaking, includes all drug-treatment, is usually restricted to certain remedial measures that are based upon experimental work, and largely founded upon Ehrlich's theory of receptor action. The difficulty of killing living parasites which have gained entrance into the body lies in the fact that most substances which are anti-parasitic are also poisonous to the tissues of the host. The great requisite, therefore, of an efficient remedy is that it shall have the power of attaching itself to the parasite and killing it (parasitotropic), while having no affinity for the cells of the body. Ehrlich proceeded on the principle that, by introducing different organic radicals into the substance containing such a parasiticide as arsenic, it might be possible to find one which would act as a copula capable of anchoring the arsenic to the parasite, while the tissues, having no suitable receptors, remained immune. The great triumph of this method of research was the discovery of salvarsan as a remedy for syphilis.

The systematic search for such chemical parasitocides was first taken up in connection with the form of *trypanosomiasis* which produces the fatal disease sleeping-sickness or African lethargy. The use of arsenious acid with some success led to the trial of other compounds of this element, of which **atoxyl** (sodium paramidophenol-arsenate) and **arsenophenyl-glycine** were the most valuable. These compounds were found to be much less toxic than arsenious acid, but in spite of this many accidents occurred from their use, especially affections of the optic nerve, which prevented their employment from becoming general.

Two peculiar features were noted in connection with the employment of atoxyl: (1) that this substance is not itself



destructive of trypanosomes *in vitro*, some vital activity being apparently needed to develop its parasitocidal properties; and (2) that, as a result of its use, the trypanosomes in an infected individual ultimately become immune to arsenic, the susceptible parasites being apparently killed and the survivors being able to propagate a race refractory to the poison.

To meet this second difficulty, attempts were made to combat the disease with an allied element, antimony, and a series of compounds of this element, homologous with the arsenical compounds which were found useful, was prepared and tried with some success; alternating courses of arsenic and antimony were administered to patients with considerable benefit, but without any certainty of resulting cure.

Some of the antimony preparations used are enumerated by Uhlenhuth, Mulzer and Hügel, viz. antimonatoxyl or sodium *p*-aminophenyl-antimoniate; sodium acetyl-*p*-aminophenyl antimoniate, equivalent to arsacetin; sodium *p*-urethane-phenyl-antimoniate, and sodium benzol-sulfon-*p*-aminophenyl-antimoniate.<sup>1</sup> Also, the application of an ointment containing "trixidine" (antimony trioxide), corresponding with the use of mercurial inunction, is commended as producing a "*therapia mite* (sic) *curans*," as opposed to Ehrlich's unattainable *therapia magna sterilisans*, by Kolle, Hartoch, Rothermundt, and Schürmann.<sup>2</sup>

Another series of drugs used for the purpose of destroying trypanosomes in infected persons was that of benzidine colours—trypan-red, trypan-blue, and so forth;<sup>3</sup> while yet another was formed of compounds of trimethylamine—malachite green, parafuchsine, and tryparosan. The results were not very satisfactory.

<sup>1</sup> *Deut. med. Woch.*, 1913, p. 393. See also Plimmer and Thomson, *Proc. Roy. Soc.*, 1908, lxxx. 1; Mesnil and Brimont, *Bull. Soc. Path. Exot.*, 1908, i, 44, 210.

<sup>2</sup> *Ibid.*, 1913, p. 825.

<sup>3</sup> Ehrlich and Shiga, *Berl. klin. Woch.*, 1904, March 28 and April 11; Nicolle and Mesnil, *Ann. Inst. Pasteur*, 1906, xx. 417, 513.



The most conspicuous research directed to finding a chemical parasiticide was that carried out by Ehrlich and his coadjutors with a view to discover an arsenical antidote to syphilis. An immense number of compounds were investigated, the most successful being the 606th of the series, subsequently known as "606" or **salvarsan**. This is chemically dioxy-diamido-arseno-benzol, of which the graphic formula is shown below :



Dioxy-diamido-arseno-benzol = "606."

#### METHOD OF ADMINISTERING SALVARSAN

Salvarsan was originally injected intramuscularly in an oily suspension. It was soon found, however, that the intravenous injection of a neutral saline solution gave better results in a shorter space of time, and this method is now universally adopted. It is, however, frequently followed during the ensuing six hours by certain unwelcome **general symptoms**, such as pyrexia (often up to 104° F.), rigors, nausea, vomiting, and diarrhœa, the majority of which can be avoided if due attention is paid to certain points in technique, such as the use of bacteria-free sterile distilled water for the preparation of the solution of the drug and for the saline diluent, and careful preparation of the patient. It should be noted that in patients who are the subjects of primary and early secondary lesions the injection of salvarsan is followed by a sharp rise of temperature during the four to six hours immediately following the injection, said to be due to the liberation of endotoxin from the bodies of the spirochætes which have been destroyed by the drug. This pyrexia is variable in extent and is unavoidable. The

appearance of a rash, or the extension of an already existent rash, may also occur a few hours after the injection and persist for several days—a phenomenon known as the Herxheimer reaction, and attributed to the same cause, or alternatively to stimulation of the growth of spirochaetes by a dose which is insufficient to cause their death. According to Rolleston,<sup>1</sup> the injection causes a fall of blood-pressure; and on rare occasions symptoms of syncope, cyanosis, imperceptible pulse, and cessation of breathing immediately follow the administration of the drug. In such cases the subcutaneous injection of adrenalin must be resorted to. The bulk of the arsenic is excreted by the kidneys within twenty-four hours, and usually the elimination is completed within a week; but occasionally it is delayed, therefore a second injection should not be given until all traces of the drug have disappeared. Several deaths from acute arsenical poisoning have been recorded through neglect of this point.

**Local symptoms**, if the injection is skilfully carried out, should be entirely absent.

**Before injection.**—The patient should be prepared for the intravenous injection as for a major operation. A sharp purge administered overnight should be followed in the morning, if necessary, by an enema. Breakfast should consist of a cup of beef-tea and a piece of dry toast only. No further food should be administered before the operation. The patient should remain in bed, and the injection be given about midday.

The general procedure is as follows:—

- (a) Distilled water and normal saline solution must be freshly prepared, sterilized, and brought to a temperature of 40° C.
- (b) Open the phial of salvarsan (by filing the neck with the file supplied in the packet, and snapping off the neck), and empty its contents into a sterilized dry flask containing sterile glass beads. Shake for a few seconds in order to break up any lumps of the drug.

<sup>1</sup> *Brit Med. Journ.*, 1915, ii. 281.

- (c) Measure out 30 c.c. of hot sterile distilled water and pour into the flask. Shake gently and evenly until the whole of the salvarsan is dissolved. An *acid* solution of salvarsan has now been prepared containing 0.6 grm. in 30 c.c., or 0.1 grm. in every 5 c.c. (the usual dose for a small infant is 0.2 grm., for a child or feeble woman 0.4 grm., for a robust woman 0.5 grm., and for a man 0.6 grm.). If therefore it is decided to give a dose of less than 0.6 grm., the surplus solution should be removed from the flask by means of a sterilized measuring pipette and discarded.
- (d) Add to the acid solution of salvarsan sufficient of the normal saline solution to make the bulk of the fluid up to 100 c.c.
- (e) Neutralize the acid solution by the addition of normal caustic soda, of which 0.7 c.c. should neutralize each 0.1 grm. of salvarsan; usually rather more is required. Assuming the dose given to be 0.6, then 4 c.c. of soda solution should be added, and will result in the formation of a copious yellow precipitate, which on the flask being gently shaken will again go into solution. If the colour of the fluid is dull, or all the precipitate has not been taken up, add soda solution drop by drop, shaking the flask gently the while, until the desired result is obtained. Only solutions sherry-bright in colour and perfectly free from flocculi should be injected.
- (f) Now make up the bulk of the solution to about 200–250 c.c. by the further addition of warm normal saline solution.

**At the time of injection.**—The strictest attention should be devoted to asepsis. The operation is carried out in the main as described under Intravenous Injection (p. 51). The fluid may be introduced with the help of a special syringe provided with a two-way tap, permitting the barrel to be refilled without removing the needle from the vein; preferably the needle used for the venipuncture is attached by a length of rubber tubing to a burette containing the salvarsan solution, and the injection made by gravity.

**After injection.**—The limb which has been operated upon—usually an arm—should be raised on a pillow and lightly covered with cotton-wool or a wrap to keep it warm. The pillow should be removed from under the patient's head. The patient should remain as still as possible for

the next twenty-four hours, and in no case rise or get out of bed. Whenever necessary the bed-pan must be used.

No solid food should be given during the twenty-four hours following the injection; milk and soda, beef-tea, Benger's food or similar preparations may be given. Twenty-four hours later, if no untoward symptoms have occurred, the patient may move in bed, sit up, have light food, fish, chicken, etc., and forty-eight hours after the injection may go on to full diet and resume his ordinary avocation.

**Neo-salvarsan.**—Still further investigation conducted up to the 914th compound led to the discovery of a compound of salvarsan with sodium-formaldehyde-sulphoxylate ( $\text{CH}_2[\text{OH}].\text{P}.\text{SO}.\text{Na}$ ), to which the name neo-salvarsan has been applied, and which is more easily administered and less liable to be followed by disagreeable effects, although not quite so effective.

The dose of neo-salvarsan up to 0.9 gram., which corresponds to 0.6 gram. salvarsan, should be dissolved in 10 c.c. of sterile distilled water or sterile normal saline solution, and slowly injected into a vein by means of an all-glass syringe. The concentration of the drug does not appear to be of importance; we have injected 0.9 gram. dissolved in 2 c.c. of water, intravenously, with good results.

#### BRITISH AND FRENCH EQUIVALENTS OF SALVARSAN

Salvarsan was marketed in England by the firm of Meister Lucius & Brüning, and with the outbreak of the present European War the supply of German-controlled "606" ceased.

The French, however, have manufactured an identical product from the time Ehrlich published his experiments, the chief supply being derived from the house of Messrs. Poulonc Frères under the name of **arseno-benzol** or **arsenobillon**. **Novo-arseno-benzol**, corresponding to neo-salvarsan, was also made by the same firm. The directions for using these preparations are identical with

those already given for the use of salvarsan and neo-salvarsan.

Recently, the Board of Trade has granted a licence to Messrs. Burroughs Wellcome & Co. to prepare the drug under the name of **kharsivan**. This is now on the market and, save that it is not so readily dissolved, is identical with the original salvarsan.

Another substitute for salvarsan is known as **galyl**. It is a derivative of arseno-benzol, two molecules of which are linked with two phosphoric groups to form tetraoxy-diphospho-amino-diarseno-benzene. It is sent out for intravenous injection as a yellow powder, in hermetically sealed glass ampoules, accompanied by a tube of sodium carbonate solution sufficient when used for the dilution of galyl to form an isotonic solution, which must be prepared immediately before use. Three injections are recommended at weekly intervals in doses of 0.25, 0.30, 0.35 or 0.40 grm. Excellent results have been obtained in the treatment of syphilis with this drug by Abraham,<sup>1</sup> Foerster<sup>2</sup> and Spence.<sup>3</sup>

**Intramine**, a sulphur di-ortho-body synthesized by McDonagh,<sup>4</sup> has not yet had a sufficiently extended clinical trial to permit of judgment being passed upon its value.

### Salvarsan in diseases other than syphilis.—

Salvarsan has been tried, and good effects attributed to it, in numerous other affections besides syphilis. In yaws (*framboesia tropica*), a single injection is said to bring about lasting cure (Castellani,<sup>5</sup> Rost,<sup>6</sup> Sabella<sup>7</sup>). In spirochætal affections of the mouth it has been recommended by Gerber,<sup>8</sup>

<sup>1</sup> *Brit. Med. Journ.*, 1914, i. 582.

<sup>2</sup> *Lancet*, 1915, ii. 645.

<sup>3</sup> *Ibid.*, 1915, ii. 1292.

<sup>4</sup> *Ibid.*, 1916, i. 236.

<sup>5</sup> *Arch. f. Schiff's. u. Tropenhyg.*, 1911, xv. 11.

<sup>6</sup> *Münch. med. Woch.*, 1912, p. 924.

<sup>7</sup> *Il Policlin.*, Sez. Prat., Aug., 1912.

<sup>8</sup> *Münch. med. Woch.*, Feb. 28, 1911.

and as a local application by Zilz,<sup>1</sup> and in the probably spirochaetal diseases, tropical ulcer and ulcerative granuloma of the pudenda, by Rodenwaldt,<sup>2</sup> Külz<sup>3</sup> (locally), and Sabella.<sup>4</sup> In malaria it is said to be effective in tertian but not in quartan or malignant cases (Iversen and Tuschinski).<sup>5</sup> Bramwell<sup>6</sup> has recorded two cases of pernicious anæmia treated with salvarsan; and we have ourselves employed the drug with benefit in several cases of pernicious and other forms of anæmia. Still other conditions in which this remedy has been tried are pellagra (Nice, MacLester, and Torrance,<sup>7</sup> Cole and Winthrop<sup>8</sup>), lichen ruber planus (Herxheimer),<sup>9</sup> rabies (Tonin),<sup>10</sup> small-pox (Sunder),<sup>11</sup> chorea (Szametz),<sup>12</sup> amœbic dysentery (Matsuura),<sup>13</sup> Oriental sore (Petersen),<sup>14</sup> and sarcoma (Czerny and Cann).<sup>15</sup>

Noguchi has now shown conclusively that general paralysis of the insane is a late manifestation of the activity of the *Treponema pallidum*, and consequently treatment of the disease has once more resolved itself into the use of active antisiphilitic remedies, chiefly on the lines of (1) intrathecal injection of salvarsanized serum (Marmisco,<sup>16</sup> Ford Robertson,<sup>17</sup> Purves Stewart,<sup>18</sup> Swift and Ellis<sup>19</sup>), and

<sup>1</sup> *Münch. med. Woch.*, 1912, p. 20.

<sup>2</sup> *Arch. f. Schiff's. u. Tropenhyg.*, 1912, xvi. 562.

<sup>3</sup> *Ibid.*, p. 563.

<sup>4</sup> *Il Policlin.*, Sez. Med., 1912.

<sup>5</sup> *Deut. med. Woch.*, 1911, No. 3.

<sup>6</sup> *Brit. Med. Journ.*, 1911, i, 547.

<sup>7</sup> *Journ. Amer. Med. Assoc.*, March 25, 1911.

<sup>8</sup> *Ibid.*, June 17, 1911.

<sup>9</sup> *Deut. med. Woch.*, 1910, No. 33.

<sup>10</sup> *Il Policlin.*, July 14, 1912.

<sup>11</sup> *Arch. f. Schiff's. u. Tropenhyg.*, 1912, xvii. 563.

<sup>12</sup> *Münch. med. Woch.*, 1912, p. 2333.

<sup>13</sup> *Abstr. in Zeits. f. Immunitätsforsch.*, 1911, p. 1020.

<sup>14</sup> *Münch. med. Woch.*, 1912, p. 2491.

<sup>15</sup> *Ibid.*, April 25, 1911.

<sup>16</sup> *Presse Méd.*, Jan. 28, 1911.

<sup>17</sup> *Edin. Med. Journ.*, 1913, pp. 293 and 428.

<sup>18</sup> *Brit. Med. Journ.*, 1914, i. 949.

<sup>19</sup> *Münch. med. Woch.*, Sept. 9, 1913.

(2) the direct injection of a small quantity of 6 per cent. solution of neo-salvarsan (Ravaut)<sup>1</sup> into the spinal canal immediately before the injection of 0·45 gm. of the drug intravenously.

The technique of the former method is as follows: Administer an intravenous injection of 0·3 gm. salvarsan or 0·45 gm. neo-salvarsan; one or two hours later withdraw 40 to 60 c.c. blood by venipuncture, and allow it to clot. Separate off the serum, dilute it with normal saline to form a 40 per cent. solution, and place for one hour in a water-bath at 56° C. Twenty-four hours later perform lumbar puncture (*see* p. 54) upon the patient, and withdraw 10 to 20 c.c. of cerebro-spinal fluid. Then run in, by gravity, 30 to 35 c.c. of the diluted serum, and elevate the foot of the bed to favour diffusion of the serum upwards to the cranial cavity. Repeat the entire process at intervals of seven to fourteen days.

#### RELAPSING FEVER

**Etiology.**—The causal agent of relapsing fever was discovered by Obermeier, and is named after him the *Spirochaeta* (*spirillum*) *obermeieri*.

**Agglutination and bacteriolysis.**—Sawtschenko and Melkich<sup>2</sup> found that the serum of convalescing patients had an agglutinative power on the organisms, and that it also contained a copula which was capable of producing destruction of the organisms within the leucocytes. They held that the action of this body was to transform the negative chemiotactic effect of the spirochaetes upon the leucocytes into positive chemiotaxis (*i.e.* opsonizing). There was no free complement in the blood-plasma, so that no bacteriolysis took place outside the cells. Heating the serum

<sup>1</sup> *Ann. de Méd.*, 1914, p. 51.

<sup>2</sup> *Ann. de l'Inst. Pasteur*, 1901, xv. 207. The bactericidal power of the blood of the convalescents was first shown by Gabritschewsky, *Ann. de l'Inst. Pasteur*, 1896.



led to loss of its bacteriolytic property, but not of its agglutinins.

Hodlmoser<sup>1</sup> finds the serum of convalescents to be lytic rather than agglutinative.

**Serum treatment.**—Löwenthal<sup>2</sup> has produced a bactericidal serum by inoculation of horses with the spirilla, and good results are claimed as the result of its use.

Dobrosrakow<sup>3</sup> also immunized horses with the defibrinated blood of patients suffering from relapsing fever, and used their serum for treatment, with apparent benefit.

**Chemotherapy.**—Buth<sup>4</sup> has treated cases of relapsing fever with salvarsan, with apparently good results.

<sup>1</sup> *Zeitschr. f. Heilk.*, xxvi., Heft 11.

<sup>2</sup> *Deut. med. Woch.*, 1898.

<sup>3</sup> Abstr. in *Centralbl. f. inn. Med.*, 1907, p. 1039.

<sup>4</sup> *Berl. klin. Woch.*, 1913, p. 1231.

## CHAPTER XXII

### MALIGNANT TUMOURS

**Etiology of malignant growths.**—Nothing certain is known as to the causation of malignant tumours. The hypothesis that they are produced by the action of parasitic organisms appears to lack any satisfactory evidence and is insufficient to account for many of the peculiarities of new growths. Sanfelice<sup>1</sup> produced some evidence in favour of the blastomycetic origin of malignant neoplasms, claiming to have produced both carcinoma and sarcoma experimentally by the injection of toxins of such micro-organisms, and to have cured these experimental tumours by the administration of an antitoxic serum prepared by immunizing other animals by the injection of attenuated toxins. But hitherto no generally accepted organism or toxin has been isolated with which an antitoxic or germicidal serum could be prepared. Treatment has, however, been tried on several different lines. On the supposition that a parasite is present in tumours, although it has not been discovered, some observers have tried injecting animals with portions of cancerous or other tumours, and applying the serum obtained from them to the cure of the disease. Reference has already been made in the introductory chapters of this work to the attempt to obtain, by injection of cancer-cells into animals, a cytolytic serum which shall be specific for these tumours : the practical results of this mode of treatment do not appear to have been very encouraging. The most hopeful line of treatment on bacterial lines is that inaugurated by

<sup>1</sup> Brit. Med. Assoc. meeting, Belfast, 1909.

Coley, who employs the toxins of certain bacteria which seem to have a power of producing degeneration in the cells of tumours ; but it need hardly be pointed out that this mode of treatment is quite different from serum treatment, or even from vaccines or toxins as applied to other diseases in which the specific organism is known.

**Diagnosis.**—Numerous serological methods for the diagnosis of malignant growths have also been proposed ; thus the complement-fixation test has been employed, an alcoholic extract of tumour-tissue serving as antigen (von Dungern),<sup>1</sup> and the antitryptic value of the serum has been estimated, an increase in this content being held to point to the existence of a tumour.<sup>2</sup> Piorkowski<sup>3</sup> states that a ring of precipitate is formed when a solution of saponified cancer-tissue is brought into contact with the serum of a patient suffering from the disease. Freund and Kaminer<sup>4</sup> report that normal human serum dissolves cancer-cells, but that the serum of a cancer-patient does not do so. The meiostagmin reaction (p. 92) has also been used for the diagnosis of malignant disease.

A special cutaneous reaction (haemolytic skin-reaction) has been described by Elsberg,<sup>5</sup> which consists in injecting intracutaneously 5 minims of a 20 per cent. suspension of normal blood-corpuscle in saline fluid. If the patient is the subject of cancer, a swollen area appears within three to twelve hours, and undergoes changes of colour from brown to greenish-yellow. In a normal person no such result occurs. Elsberg, Neuhof and Geist<sup>6</sup> found the reaction present in 62 per cent. of patients who were the subjects of malignant disease, and only in 15 per cent. of non-cancerous persons. Advanced cases with multiple metastases

<sup>1</sup> *Münch. med. Woch.*, 1912, p. 65.

<sup>2</sup> Müller and Jochmann, *Verhandl. Kongr. inn. med. Wiesb.*, 1907.

<sup>3</sup> *Berl. klin. Woch.*, 1914, No. 6.

<sup>4</sup> *Wien. klin. Woch.*, 1910, No. 34.

<sup>5</sup> *Journ. Amer. Med. Assoc.*, March 27, 1909.

<sup>6</sup> *Amer. Journ. Med. Sci.*, Feb., 1910.

are, however, invariably negative. Risley,<sup>1</sup> on the other hand, finds the numbers only  $33\frac{1}{3}$  per cent. in cancerous subjects, and 20 per cent. in normal individuals; hence the test seems of little value.

#### COLEY'S FLUID

**Principle of treatment.**—It was observed many years ago that an attack of erysipelas occurring spontaneously in a patient suffering from malignant disease sometimes had the effect of causing a disappearance or retrogression of the growth. Attempts were therefore made to treat cases of cancer by artificial production of erysipelas, by inoculation with the streptococci. Some very successful results were obtained by Fehleisen both in sarcoma and carcinoma, and Coley and others also reported cases apparently cured by this means. Some fatal cases, however, occurred from this treatment, and it was abandoned as too dangerous. Coley<sup>2</sup> subsequently proposed to produce the same effect, but in a manner more under control, by means of injections of the toxins formed by the cocci in artificial media.

**Preparation of the fluid.**—Coley at first obtained the toxins by growing together, in flasks of broth, cultures of the *Streptococcus erysipelatosus* (*Streptococcus pyogenes*) and of the *Bacillus prodigiosus*, sterilizing by heating to  $58^{\circ}$  C., and using the unfiltered fluid, containing the dead bodies of the organisms, for injection. For weakly patients and children Coley advised the use of the filtered culture, as being less toxic (in the proportion of 1 : 10).

Now, however, the fluid is prepared in a different manner.<sup>3</sup> The streptococcus is grown in broth for three weeks. To every 100 c.c. of the culture are added 30 c.c. of an emulsion of *B. prodigiosus* (standardized by Kjeldahl's method to contain 12.5 mg. of protein per c.c.) and 20 c.c.

<sup>1</sup> *Boston Med. and Surg. Journ.*, 1911, ii. 127.

<sup>2</sup> Art. "Erysipelas, Curative," in Quain's "Dictionary of Medicine," 3rd ed., 1902, p. 486.

<sup>3</sup> *Proc. Roy. Soc. Med.*, Surgical Sect., 1909, July 13.

glycerin. A piece of thymol is added to the mixture, and the whole is sterilized by heating to 75° C. for two hours. Virulent strains of the streptococci are used for making the cultures, their vigour being maintained by passage through rabbits.

**Dose of the fluid.**—The initial dose advised by Coley is  $\frac{1}{6}$  to  $\frac{1}{4}$  minim, injected into the buttock with all anti-septic precautions; if injected into the substance of the tumour, the dose should not be more than  $\frac{1}{16}$  minim. The injections may be repeated every two or three days. A temperature reaction of about 102°–104° F. should be aimed at, and, so long as this is obtained, no increase in the dose should be made. When necessary, the increment should be  $\frac{1}{4}$  minim. The biggest dose is usually 8 minims, though 20 minims have been given. If no good results are observed within the first three or four weeks, it may be concluded that the case is not suitable for the treatment. If, however, any diminution in the size of the growth is seen, the injections may be continued over long periods of time, until the tumour has entirely disappeared. Even then the injections should be continued in smaller doses at longer intervals for another four months. Occasional intermissions in the course of treatment are advisable.

**Results of injections.**—Coley<sup>1</sup> reported a total of 230 cases in which his treatment had been adopted, and among these 2 died as a result of the injections—one from accidental sepsis (staphylococcic), the other from embolism due to too large an initial dose. Thirteen patients had passed the three-year limit, generally considered to authorize the claim of “cure.” More recently<sup>2</sup> he has reported a further series of cases, making the total up to 500, and including one more death from embolism. It also includes 52 inoperable cases which were successfully treated, and of these 35 remained well after from three to sixteen years. Recurrences took place in 3 cases which had shown

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1900, i. 906.

<sup>2</sup> *Op. supra cit.*

definite improvement, thus attesting the correctness of the diagnosis. Coley also records 35 cases treated by other surgeons, with the result that in 26 the tumours disappeared, and 14 patients were alive from two to four years afterwards.

In other hands the treatment has not always proved so successful as would appear from Coley's results, but it may be pointed out that many of the failures date from fifteen years ago.

The cases best adapted for this toxin treatment would seem to be the spindle-celled sarcomas, which are those least malignant in type and most nearly approaching organized tissue in their structure. Coley<sup>1</sup> collected 430 cases of sarcoma treated by this fluid - the tumours entirely disappeared in 47 (11 per cent.), and 28 of these cases were alive and well after from three to fifteen years. Melanotic sarcoma does not seem amenable to the injections. Coley does not recommend his treatment in cases in which operative removal of the growth is possible. In cases which are inoperable it appears well worthy of trial.

Coley explains the good effects seen after injection of toxins as being due to the induction of an environment unsuitable to the life of the cancer-cell, which degenerates in consequence. He holds that malignant tumours are produced by a parasitic organism which is affected by this treatment, just as cases of tuberculosis and of syphilis have been observed to show improvement after attacks of erysipelas. It does not appear necessary to see in Coley's results a support to the parasitic hypothesis. It is well known that the cells of tumours are of low vitality; and it is quite conceivable that they may succumb to the action of poisons circulating in the blood, when more resistant cells, such as those of normal tissues, are unaffected. It is also possible that the effect of the toxins is to supply in some way a stimulant to the normal connective tissue, and that its cells are enabled to offer a more vigorous resistance to the invasion of the tumour as the result of this

<sup>1</sup> *Boston Med. and Surg. Journ.*, 1908, clviii. 175.

stimulus. In tuberculosis and syphilis the action of erysipelas must be exerted in the direction of an increase of tissue-reaction or possibly of phagocytosis, since it can hardly be maintained that the toxins of streptococci have a specific action on other organisms. The effect of bacterial toxins in inducing granular and fatty degeneration of tissues is well recognized, and the proneness of the cells of tumours to undergo these changes is noteworthy. That a conflict takes place between the healthy cells of the body and the invading cells of a tumour seems evident, not only on theoretical grounds, but on account of the signs of irritation and reaction seen at the periphery of a malignant growth. It is not impossible that both of the factors suggested may play a part in the action exerted by the toxins of erysipelas on tumours. It is noteworthy that the most marked effect is produced on the sarcomas, which are connective-tissue tumours, and that the reaction which is called "inflammation," and which is induced by the action of bacterial toxins, is also seen in connective tissue.

# SERUM TREATMENT

**Emmerich and Scholl's serum.**—An attempt on rather different lines to utilize erysipelas as a cure for tumours was made by Emmerich and Scholl,<sup>1</sup> who inoculated sheep with the cocci of erysipelas, and used the serum of these animals for treatment of patients. Improvement seemed to result in some cases, but not actual cure. Reineboth<sup>2</sup> records a case in which this serum was used, and in which the growth showed signs of softening as a result of the injections; but the patient died in spite of the treatment.

**Wlaeff's serum.**—Wlaeff and D'Hotman de Villiers<sup>3</sup> obtained cultures of blastomycetes from cancerous growths, and with them inoculated pigeons. They then took the serum of these birds and tried it on rats as a protective

<sup>1</sup> *Deut. med. Woch.*, 1895, No. 17.

<sup>2</sup> *Ibid.*, 1895, No. 48.

<sup>3</sup> *Compt. Rend. de la Soc. de Biologie*, 1900, p. 611.



against the form of cancer from which these rodents suffer. This serum has been used as a remedy for human cancer. Wlaeff reports that it causes the leucocytes to surround, penetrate, and destroy isolated epithelial cells. Reynier<sup>1</sup> reports that its use relieves pain and produces general improvement in patients, but that the growth of the tumours is not checked. Other cases with very similar results are recorded by Berger<sup>2</sup> and Richelot.<sup>3</sup> Lucas-Championnière<sup>4</sup> did not find that any benefit was derived from the injections in cases in which he tried it.

The idea that blastomycetes are the causal agents in cancer is not maintained by many authorities at the present day, and it is difficult to believe that any real effect can be produced in human cancer by such a serum as that just described. It is possible that a certain degree of irritation might be caused by foreign serum injected into a human being, and that some temporary effect might be induced in a tumour into which it was injected. Probably the only good results were owing to suggestion; the patient was led to believe that some good would be done, and either imagined this or attributed some incidental improvement to the serum.

**Doyen's antitoxic serum.**—Doyen<sup>5</sup> has isolated from malignant growths an organism to which he gives the name of *Micrococcus neoformans*, and has prepared an antagonistic serum by injection of its toxins into animals. This serum he has employed for the treatment of cancer, injections being made into the buttocks. Of a total of 126 cases, 58 showed no improvement, 18 cases were described as on the way to cure, 29 more had improved under the treatment, and in 21 cases the growth had completely disappeared. The serum was tried by

<sup>1</sup> *La Semaine Méd.*, 1901, p. 59.

<sup>2</sup> *Ibid.*, 1901, p. 69.

<sup>3</sup> *Ibid.*, 1902, p. 142.

<sup>4</sup> *Ibid.*, 1900, p. 410.

<sup>5</sup> *La Presse Méd.*, 1904, No. 16 (*Trans. Soc. de Biol.*).

Kirmisson<sup>1</sup> and by Morgan and Paine<sup>2</sup> with negative results.

**Schmidt's serum.**—Schmidt,<sup>3</sup> of Cologne, prepared a serum by inoculating horses and sheep with cultures of a parasite derived from malignant growths, to which the names "cancroidin" and "antimeristem" have been applied. He claims good results from this preparation, but states that still greater benefit is derived from inoculation of the actual organisms themselves. The use of such a *vaccine* is followed by a reaction at the site of the growth, with some accompanying rise of temperature. The growth of the tumour is arrested, and retrogressive changes take place in the cells of which it consists. Schmidt maintains that the parasites isolated by other observers are all different forms of one organism, which is pleomorphic, and assumes different appearances according to its conditions of culture. Trial was made of Schmidt's serum in 9 cases at the Middlesex Hospital;<sup>4</sup> it was not found to influence the course of the disease. Stockmann<sup>5</sup> found that its use increased pain and produced no apparent benefit, while it was very expensive. Kolb<sup>6</sup> also found it quite inefficacious.

**Cytolytic serum.**—Dubois,<sup>7</sup> who as early as 1897 injected macerated tumours into animals, and used the serum as a remedy, reported that fibrosis was thereby induced in new growths. Leyden and Blumenthal<sup>8</sup> endeavoured to prepare a cytolytic serum by injecting rabbits with finely

<sup>1</sup> *La Semaine Méd.*, 1905, p. 361.

<sup>2</sup> *Lancet*, 1906, i. 955.

<sup>3</sup> An account of Schmidt's work was given by Dr. H. J. Johnson at the Abernethian Society of St. Bartholomew's Hospital, Nov. 5, 1903. (See *Lancet*, 1903, ii. 1374.)

<sup>4</sup> *Lancet*, 1904, i. 684. Cf. Power (*Brit. Med. Journ.*, 1904, i. 299), who found that the serum produced an inflammatory reaction but did not influence malignant growths.

<sup>5</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1913, p. 286.

<sup>6</sup> *Berl. klin. Woch.*, 1912, No. 17.

<sup>7</sup> *Rev. Méd. de l'Est*, Feb. 1, 1897.

<sup>8</sup> *Deut. med. Woch.*, Sept. 4, 1902.

divided tumours taken from dogs. They considered that they had good results in dogs affected with tumours, which received injections of the serum of the rabbits thus prepared. The serum was subsequently tried on human patients, carcinoma-cells from human sources being used for the preparation of the serum. The writers state that benefit was derived from the injections in some inoperable cases.

J.-B. Charcot<sup>1</sup> tried a similar serum, but admits that the results obtained were open to question. Some local reaction was produced by the serum at the seat of the tumour. The injections were not painful.

With regard to the use of serums of this nature, we are met by the difficulty that at present no proof is available that the cells of an epithelial tumour are of a different nature from those of the normal epithelium from which they are derived. Hence it is impossible to know whether it is practicable to produce effects on the cells of the tumour without the simultaneous occurrence of destructive changes in normal cells. The best hope in this respect seems to lie in the recognized lack of resistance met with in the cells of tumours, which, in spite of their rapid growth, or perhaps in consequence of it, are liable to undergo early degeneration. It is possible that a weak destructive force, such as might be supplied by an epitheliolytic serum, might suffice to kill tumour-cells, while unable to affect injuriously cells of normal resistance. The effects of the cytolytic serums at present prepared do not appear to be very potent.

#### CANCROÏN AND SIMILAR PREPARATIONS

Adamkiewicz<sup>2</sup> has prepared an extract of cancers with which he claims to have had astonishingly good results in cases of cancer. The extract is said to consist principally of neurine with some preservative fluid. He reported cases of cancer of the tongue, œsophagus, stomach, larynx,

<sup>1</sup> *La Semaine Méd.*, 1900.

<sup>2</sup> *Berl. klin. Woch.*, 1902, No. 24.

and breast in which great improvement was effected by his preparation; and Kretzmer<sup>1</sup> records another case of œsophageal cancer which rapidly improved under injections of cancroïn.

Very severe criticisms of the cases recorded by Adamkiewicz were made by Nothnagel and others. It was pointed out that the diagnosis of cancer was not definitely made in any one of the cases. Carcinoma of the stomach is a condition which it is very difficult to diagnose with certainty, and the patient who was said to be suffering from this disease and to be benefited by the cancroïn subsequently came back again for treatment for vomiting. In cases of cancer of the œsophagus, pieces of the growth may at times slough off and so leave a passage for food, rendering swallowing once more possible for a period of time. This may have occurred in the cases recorded above, and the temporary benefit have been ascribed to the cancroïn.

Poten<sup>2</sup> failed to obtain any improvement in two cases (cancer of breast and of uterus) in which he employed cancroïn. He points out that it is of no use to record cases of improvement under this or any other remedy unless the diagnosis is confirmed by microscopical evidence.

It is almost impossible to believe that a remedy of the composition assigned to cancroïn can have any real effect on malignant growths. Much more definite evidence than that at present available will have to be forthcoming before the claims of this cure for cancer can be taken seriously.

An extraordinary variety of **cellular preparations** have been tried in cancer. Thus Vaughan<sup>3</sup> used a residue left after extraction of ground-up cancer-cells with water, saline solution, alcohol, and ether; he believed that thereby the growth of some superficial tumours was checked and pain diminished. Rovsing<sup>4</sup> administered a fluid expressed from

<sup>1</sup> *Petersburg. med. Woch.*, 1902, No. 20.

<sup>2</sup> *Berl. klin. Woch.*, 1902, No. 28.

<sup>3</sup> *Med. Record*, 1910, lxxvii. 892.

<sup>4</sup> *Ibid.*, 1911, lxxix. 29.

the patient's own tumour, and thought he saw beneficial results in 2 cases. Gilman and Coca, at the Philippine General Hospital, used emulsions of living cancer-cells; but Risley<sup>1</sup> found that, so far from checking the growth, such treatment might even accelerate it. Ill and Minningham<sup>2</sup> employed injections of ascitic fluid from a case of cancer affecting the peritoneum, and noted some subjective improvement—this seems to occur with almost any remedy which excites new hopes—but no cases of cure.

### VACCINE TREATMENT

Doyen is perhaps alone in regarding the *Micrococcus neoformans* as the ultimate cause of malignant growths, but it is an undoubted fact that this variety of staphylococcus is very frequently associated with secondary suppurations, and the use of a vaccine in doses of 100 to 250 millions is, under such conditions, of considerable value. Jacobs and Geets<sup>3</sup> treated 37 cases of carcinoma of the breast thus, with more or less improvement in 26.<sup>4</sup>

**Summary.**—The most remarkable fact about the various serums and remedies above alluded to is that they all seem to have produced good results in the hands of their inventors, but few, if any, of them appear to have succeeded in those of others. The only one which can be said to have established any pretence to efficacy is Coley's fluid, which has been tried now in a sufficient number of cases to afford material for forming a judgment. It appears to be definitely established that erysipelas may cause the disappearance of malignant growths, especially sarcomas; and the records of cases treated by Coley's toxins give ground for hope that the use of the toxins may be followed by similar good effect without the dangers of the actual

<sup>1</sup> *Boston Med. and Surg. Journ.*, Nov. 23, 1911.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, Aug. 17, 1912.

<sup>3</sup> *Lancet*, 1906, i. 964.

<sup>4</sup> See also above, under "Schmidt's serum," p. 429.

inoculation with bacteria. The treatment is certainly worthy of trial in cases which are beyond the aid of the surgeon. It is most important, however, to recognize that no time should be lost in medicinal treatment of any kind, if there is a possibility of removing the growth with the knife. This remains at present the only method which holds out reasonable hopes of cure in malignant disease.

### CONCLUSIONS

1. Coley's fluid may be tried in inoperable cases of malignant disease, especially of sarcoma. It should not be used as a temporizing measure in cases which are amenable to surgical interference.

2. Until it is proved that cancer is an infective disease, serum treatment of the usual kind is inapplicable to this condition. It is, however, permissible to hope that it may be possible to produce a cytolytic serum which may act on the cells of the growth without affecting the normal cells of the tissues. A satisfactory serum of this nature does not appear to have been as yet prepared.

3. In secondary suppurative infections of malignant growths a vaccine of the responsible organisms—whether *Micrococcus neoformans*, or other bacteria—will frequently relieve certain of the more distressing symptoms.

## APPENDIX

### VARIOUS CONDITIONS TREATED WITH SERUMS, ETC.

#### THERAPEUTIC USE OF NORMAL HORSE-SERUM, ETC.

MONTGOMERY PATON,<sup>1</sup> as a result of trial of diphtherial antitoxic serum in diseases other than diphtheria, was led to believe that the serum or plasma of normal (untreated) animals had a definite effect in raising the power of resistance to infective conditions. He prefers, however, the serum of horses which have undergone a slight degree of immunization against diphtherial toxins, as by this procedure, which acts by raising the resistance of the tissue-cells, the stimulating effect of the serum is increased. His work in cases due to pyogenic infection has already been mentioned (p. 359). He finds the use of normal plasma also valuable in tuberculosis, arthritis deformans, broncho-pneumonia, dysmenorrhœa, nephritis, cerebro-spinal meningitis, epilepsy, Graves's disease, traumatism, and other conditions. The serum is given by the mouth. The list of diseases for which it is useful is rather too long and varied for the claims made on its behalf to be very convincing.

Schmidt<sup>2</sup> and Borchardt<sup>3</sup> have injected normal serum into the peritoneal cavity before *operations* on the abdomen, with a view to increasing the resistance to any accidental septic infection; and Stuart Low<sup>4</sup> suggests the use of serum as a *dressing for wounds*. Hort<sup>5</sup> advises the use of

<sup>1</sup> "New Serum Therapy." London, 1906.

<sup>2</sup> *Deut. med. Woch.*, 1904, No. 49. <sup>3</sup> *Ibid.*

<sup>4</sup> *Lancet*, 1907, i. 1221. <sup>5</sup> *Ibid.*, 1908, ii. 462.



serum, given by the mouth, in cases of *gastric ulceration*, and H. D. Rolleston confirms his results so far as relief of pain and of hæmorrhage is concerned.

Latham<sup>1</sup> has used horse-serum by the mouth in cases of *pulmonary tuberculosis* along with tuberculin, and attributes good effects to the remedy. We have not been able to satisfy ourselves, however, that the serum has any definite value in this disease.

Weil<sup>2</sup> made trial of injections of the serum of normal individuals and of animals in cases of *hæmophilia*, with a view to supplying the coagulating substance which is wanting in these patients, and found that improvement resulted. His observations are confirmed by Broxa,<sup>3</sup> who states that normal serum may be given to these patients as a prophylactic against attacks of bleeding, and finds that diphtherial antitoxic serum, which is generally at hand, is effective. Ridder found serum treatment useless in a family of bleeders.<sup>4</sup>

It is worthy of note that Cole and Smirnow<sup>5</sup> find experimentally that foreign serum may in some instances favour infection. Thus, if the serum of pigeons be injected into mice along with cultures of pneumococci, the animals succumb more readily to the parasite, though pigeons under normal circumstances are immune to the pneumococcus.

Clars<sup>6</sup> has used diphtherial antitoxic serum in *purpura hæmorrhagica*, followed by normal horse-serum. Bigelow<sup>7</sup> uses fresh rabbit's serum in the hæmorrhagic disease of new-born infants. Dewar<sup>8</sup> believes that it was of benefit in a case of hæmoptysis. Levison<sup>9</sup> recommends the local

<sup>1</sup> *Proc. Roy. Soc. Med.*, 1908.

<sup>2</sup> *Compt. Rend. Acad. des Sci.*, cxli., Nos. 15, 17.

<sup>3</sup> *Med. Klinik*, 1907, p. 1445.

<sup>4</sup> *Charité Ann.*, 1910.

<sup>5</sup> *Johns Hopkins Hosp. Bull.*, Sept., 1908, p. 249.

<sup>6</sup> *Med. Record*, 1910, lxxvii. 642.

<sup>7</sup> *Journ. Amer. Med. Assoc.*, July 30, 1910.

<sup>8</sup> *Brit. Med. Journ.*, 1909, ii. 1664.

<sup>9</sup> *Journ. Amer. Med. Assoc.*, March 8, 1913.

application of horse-serum in cases of bleeding after operation.

#### MORPHINE ANTITOXIN, ETC.

It was stated in the first chapter of this book that the ordinary mineral and vegetable poisons, such as arsenic or morphine, do not give rise to the formation of antitoxins; and this is the view generally held. Hirschlaff,<sup>1</sup> however, claimed to have succeeded in producing an antitoxic serum which would counteract poisoning by morphine. He injected gradually increasing doses of the alkaloid into rabbits, and obtained their serum. He then ascertained exactly the minimal lethal dose of morphine for a rabbit per kilogram of body weight, and found that, if some of the serum was administered along with the poison, the animals were able to survive a larger amount of the poison than if no serum was given. The serum was also capable of protecting mice against the poison. Further, Hirschlaff found that if an emulsion of brain-substance was mixed with the morphine, larger doses of the latter could be tolerated; from this he concludes that the brain-cells have a power of fixing this poison similar to that which they exhibit towards the toxin of tetanus. In a (human) case of morphine poisoning which he observed, Hirschlaff made use of some of the antitoxic rabbit's serum, and considered that it had a distinct antidotal action. He also found it useful in enabling patients who have formed a morphine habit to leave off the drug at once, the severe nervous symptoms which usually occur in such circumstances not being experienced.

Hirschlaff's statements were severely criticized by Morgenroth,<sup>2</sup> and it is probable that his conclusions were founded on errors of observation.

An *anti-alcoholic serum* has been prepared by Sapelier and Dromard, but at present it is difficult to take the claims

<sup>1</sup> *Berl. klin. Woch.*, Dec. 8 and 15, 1902.

<sup>2</sup> *Ibid.*, 1903, No. 21.

of the preparation very seriously.<sup>1</sup> The effects of serum derived from herbivorous animals in cases of *strychnine poisoning* have been investigated by Lo Monaco,<sup>2</sup> who suggests the use of diphtherial antitoxin in this condition.

#### ANTIABRIN SERUM

Jequirity has been used in ophthalmic practice to induce inflammation in eyes which are affected with chronic indolent conditions (pannus, etc.). Difficulties frequently arise owing to the impossibility of graduating the amount of inflammation produced. It has been found possible, however, by instillation of a serum<sup>3</sup> prepared by injecting animals with abrin, the active principle of jequirity, to control the inflammatory reaction produced by preparations of this plant.

#### GRAVES'S DISEASE (EXOPHTHALMIC GOITRE)

In this disease the principle upon which serum treatment is founded is different from that in most of those previously considered, since the serum is used to neutralize a poison formed by the cells of the patient himself, not by any parasitic organisms. The treatment is in reality of the nature of "organotherapy"—administration of animal organs or extracts. Since, however, it is serum which has been used in some cases, it demands notice here.

**Etiology.**—The causation of this disease is not well understood. Serum treatment is suggested on the ground that in all probability the disorder is due to an auto-intoxication caused by an excess of the secretion normally formed by the thyroid gland. The enlargement of this structure is a frequent, though not an invariable, feature of the disease, which may apparently exist in an abortive form, lacking

<sup>1</sup> An abstract of the authors' observations may be found in the *Journ. de Méd. et de Chir. Pratiques*, June 25, 1903, p. 461.

<sup>2</sup> *Arch. Ital. de Biologia*, June 10, 1903.

<sup>3</sup> See Dieudonné, "Immunität, Schutzimpfung u. Serumtherapie," p. 121. Leipzig, 1903.

any one or even two of its main features (tachycardia, exophthalmos, and enlargement of the thyroid gland).

**Lanz's milk treatment.**—The first experiments in the direction of the treatment of Graves's disease by means of preparations derived from animals which had had the thyroid gland removed were made by Lanz,<sup>1</sup> who performed thyroidectomy on goats, and treated cases of exophthalmic goitre with their milk. He has recorded altogether six cases in which this treatment alone was adopted, and states that the results were very encouraging.<sup>2</sup> As an instance, we may quote the case of a woman aged 38, who exhibited the characters of the condition in a very marked degree. There were wasting and tremor, enlargement of the heart and tachycardia (160 per minute), and œdema of the lower limbs. Exophthalmos was well marked and von Graefe's sign present. The characteristic thrill was perceptible over the thyroid gland, which was much enlarged. The patient was very weak and depressed, and slept badly. The milk treatment was carried out in hospital for five weeks, at the end of which time very marked improvement had occurred. The woman felt much stronger, and could now walk about, which she could not do before. She was supplied with a goat from which the thyroid gland had been removed, and continued the treatment at home. At the end of a year she was apparently quite cured. Lanz states that he had never seen a cure effected previously in a patient who had reached the stage of the malady presented by this case.

Goebel<sup>3</sup> also has recorded a case of cure with the milk treatment, but as the patient was given arsenic as well it is impossible to be certain that the good result was due to the milk.

A desiccated milk derived from thyroidectomized animals has been prepared by Burghart and Blumenthal, under the name of "rodagen." It is said to be efficient,

<sup>1</sup> *Correspondenzblatt f. Schweizer Aerzte*, 1899, No. 23.

<sup>2</sup> *Münch. med. Woch.*, 1903, p. 146.

<sup>3</sup> *Ibid.*, 1902, No. 20.

but acquires an unpleasant cheesy smell and taste on keeping (Moebius). Personal trial and observation do not suggest that it is very effective.

**Serum treatment.**—Ballet and Henriquez<sup>1</sup> and Burg-hart and Blumenthal<sup>2</sup> have used the serum of dogs from which the thyroid gland had been removed, for the treatment of the malady, and they all record good results. Moebius<sup>3</sup> has made use of the serum of rams which had been similarly treated. Hypodermic injection was first employed, but was given up as unsatisfactory ; and administration by the mouth was substituted. Moebius gives 5 c.c. of the serum every other day in a tablespoonful of wine. He noted a rapid diminution in the size of the thyroid gland in his patients, with diminished frequency of the pulse and cessation of the tremor. No ill effects were observed.

Schultes<sup>4</sup> also tried this remedy, giving smaller doses at shorter intervals (0·5 c.c. three times a day). These amounts were gradually raised by daily additions of 0·5 c.c. to each dose, till the patients were taking 4·5 c.c. three times a day. Schultes first used sherry as a vehicle, and afterwards raspberry syrup. He noted the same good results as were claimed by Moebius, the pulse falling from 142 to 90 beats a minute, and the circumference of the neck diminishing to almost normal proportions.

Dreschfeld<sup>5</sup> records his experience of the serum in 21 cases, of which 10 were cured and 6 improved. He gave doses of 10 minims by the mouth thrice daily, gradually raising the dose to 20–25 minims.

Hallion<sup>6</sup> has used a glycerinated preparation of the

<sup>1</sup> *Semaine Méd.*, 1895, p. 329.

<sup>2</sup> *Deut. med. Woch.*, 1899, Nos. 37, 38. V. Leyden's "Festschrift," p. 251; Berlin, 1902.

<sup>3</sup> Schmidt's *Jahrbuch d. ges. Med.*, cclxxiii. 43; *Münch. med. Woch.* 1903, No. 4, p. 149.

<sup>4</sup> *Münch. med. Woch.*, 1902, No. 20, p. 834.

<sup>5</sup> *Med. Chron.*, 1906, xliv. 203.

<sup>6</sup> *La Presse Méd.*, Nov. 1, 1905.

whole blood of thyroidectomized animals (hæmatoéthyrine), and a preparation known as "thyroidectin" is of somewhat similar constitution.

Stradiotti<sup>1</sup> prepared a *thyrotoxic serum* by injecting a sheep with powdered thyroid glands, and used it for the treatment of patients suffering from Graves's disease, the doses being from 5 to 45 c.c. Subjective improvement and diminution of the tremor resulted, but the cardiovascular condition was not influenced.

In America a serum prepared by Beebe is in use; it is made by injecting an extract of finely powdered thyroid glands into animals, the extract containing nucleo-proteins and globulins. The serum is standardized by measuring its power of agglutinating small fragments of thyroid gland.<sup>2</sup>

The exact mode in which the milk or serum acts is not quite clear. That it must somehow counteract the excess of thyroid secretion is evident; but it can hardly be supposed that the mere injection of so small a quantity of serum free from this substance could reduce the proportion of it present in the blood of a patient suffering from Graves's disease to a point below that at which toxic symptoms occur. There must be some actively antagonistic principle in the serum or milk of the thyroidectomized animal which acts as antitoxin. It appears that symptoms of intoxication occur both when there is defect and when there is excess of thyroid secretion; poisonous substances are set free which neutralize each other in health. In other words, the function of the thyroid gland must be the preparation of a substance which neutralizes a poison formed by the other tissues of the body in the course of their activity. It would, however, be premature to form definite conclusions as to the etiology of Graves's disease on the strength of the very small number of cases which are at present available as evidence of the curative properties of the serum of

<sup>1</sup> *Riv. Crit. di Clin. Med.*, 1907, Nos. 7 and 8.

<sup>2</sup> *Journ. Amer. Med. Assoc.*, Sept. 1, 1906.

thyroidectomized animals. From the practical point of view, even should the treatment be proved successful, there would still remain the serious consideration of expense in connection with it. Moebius records that in one of his cases the cost worked out at 400 marks (£20).

Burkard<sup>1</sup> gave *diphtherial antitoxin* in doses of 3,000 units, and believed that it had a good effect. A similar observation was made by Legge.<sup>2</sup>

### LEUCHÆMIA

This disease, characterized by anæmia (defect of red corpuscles), with immense increase in the number of white corpuscles, by enlargement of the spleen and of the lymphatic glands, and sometimes by the appearance of masses of lymphoid tissue in the internal organs, is of uncertain origin. It is allied on the one hand to the group of blood-diseases, including pernicious anæmia and splenic anæmia, and on the other hand to Hodgkin's disease (lymphadenoma) and the various tumours.

Injections of tuberculin have been used in a few cases, apparently with some diminution in the number of leucocytes (Henck, Beitzke). Rolleston has also administered antistreptococcic serum in one case with similar effect, but the result on the general condition of the patient was inconclusive.<sup>3</sup>

Leucatello and Malon<sup>4</sup> have used a leucolytic serum obtained by injecting animals (rabbits, sheep) with leucocytes. They record good effects, seen in diminished number of leucocytes and shrinking of the spleen.

Larrabee<sup>5</sup> tried Coley's mixed toxins, and believed the fluid had some palliative effect.

<sup>1</sup> *Journ. Amer. Med. Assoc.*, 1906, Nov. 3.

<sup>2</sup> *Ibid.*, April 22, 1905.

<sup>3</sup> See Susmann, "Leuchæmia and Tuberculosis," *Practitioner*, 1903, ii. 541.

<sup>4</sup> *Gaz. degli Ospedali*, 1903, No. 11.

<sup>5</sup> *Med. Record*, 1911, lxxx. 152.



Recovery of an apparently moribund case of this disease under the use of salvarsan is recorded by Perussia.<sup>1</sup>

### HÆMOGLOBINURIA

The nature of this disease was investigated by Landsteiner and Donath,<sup>2</sup> who ascertained that there was present in these patients a hæmolytic amboceptor which only acted in the cold—an unusual condition of action, as heat is commonly needed for the occurrence of immunity-reactions. This discovery has been confirmed by Kumagai, Taizo, and Inoue,<sup>3</sup> and by Matsuo,<sup>4</sup> as well as by Eason,<sup>5</sup> who was credited in the last edition of this book with the original observation, as no mention of previous workers was made in his paper. The Wassermann reaction is positive in a considerable percentage of these patients, suggesting a connection with syphilis.

Widal and Rostanne<sup>6</sup> believe that in this disease hæmolysis occurs owing to the lack of an *antisensibilisatrice* (anticopula) which is present in the blood of normal persons. They tried as a remedy the serum of a rabbit which had been injected with human blood—with a view to increasing the formation of this body—and believed that good resulted from this mode of treatment.

### HODGKIN'S DISEASE

Fränkel and Much<sup>7</sup> believe that a bacillus found in the lesions is the cause of this malady. It is a Gram-positive diphtheroid bacillus, some colonies in cultures taking a coccoid form. Billings and Rosenow<sup>8</sup> prepared a vaccine

<sup>1</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1912, p. 600.

<sup>2</sup> *Münch. med. Woch.*, Sept. 6, 1904.

<sup>3</sup> *Deut. med. Woch.*, 1912.

<sup>4</sup> *Deut. Arch. f. klin. Med.*, 1912, cvii, 325.

<sup>5</sup> *Scot. Med. and Surg. Journ.*, May, 1906.

<sup>6</sup> *Compt. Rend. Soc. Bio.*, 1905, lviii, 321, 372.

<sup>7</sup> *Zeitschr. f. Hyg.*, 1910.

<sup>8</sup> *Journ. Amer. Med. Assoc.*, 1913, lxi, 2122.

of this organism, and state that they obtained good results in treatment of cases of Hodgkin's disease therewith.

## KALA-AZAR

The use of salvarsan in this disease was followed in one case by general improvement, with fall of fever, cessation of pain, and shrinking of the enlarged spleen (Caryophyllis and Sotiriades).<sup>1</sup>

## ORIENTAL SORE

Row<sup>2</sup> grew the causal organism, *Leishmania*, on Nicolle-Novy-McNeal medium, killed the cultures with glycerin, and used the dead culture as a vaccine. He records good results as obtained in three cases.

## PELLAGRA

This disease, characterized by weakness of the limbs, ending in paralysis, with peculiar mental symptoms, is, according to the Illinois Commission, "due to infection by some living organism," but its exact etiology is at present unknown. Many observers believe it is due to the use of diseased maize as food, as the poison contained in this substance can be extracted, and is capable of causing the death of animals. Babes and Manicatide<sup>3</sup> found that if the extract was injected along with blood-serum from normal persons, the fatal effect was not prevented; but that if serum from those convalescent from the disease were used, it protected the animals from the effects of the poison. An antitoxic substance is therefore contained in the serum of convalescents.

Tizzoni,<sup>4</sup> who regards a streptobacillus which he isolated from maize, and also from the blood and tissues of pellagrins, as the cause of the condition, states that an anti-hæmolysin

<sup>1</sup> *Deut. med. Woch.*, 1911, No. 41; 1912, No. 34.

<sup>2</sup> *Brit. Med. Journ.*, 1912, i. 540.

<sup>3</sup> *La Semaine Méd.*, 1900, p. 279.

<sup>4</sup> *Centralbl. f. Bakt., Orig.*, lxvii. 175.

is present in the serum of patients suffering from pellagra, which is absent from normal serum, and uses a test based on this observation for the purpose of diagnosis. Salvarsan has been tried in cases of pellagra, but its efficacy is doubtful.<sup>1</sup>

### HYDATID DISEASE

**Precipitation tests.**—Fleig and Lisbonne<sup>2</sup> found that the fluid contained in a hydatid cyst gave a precipitate when mixed with the serum of a patient suffering from the disease. This observation is confirmed by Welsh and Chapman,<sup>3</sup> who use this reaction as a means of diagnosis: 12 drops of serum should be added to 1 c.c. of the fluid. Different specimens of hydatid fluid vary in their power of giving the reaction, and careful selection of a suitable sample must be made if the phenomenon is to be used as a test for the presence of the parasite. If the reaction persists after operative removal of a cyst, it is probable that other cysts remain behind; but disappearance of the reaction does not prove the disease to be cured.

**Complement-fixation.**—The use of this reaction as a test for the presence of hydatid cyst was first suggested by Ghedini.<sup>4</sup> The antigen employed is either the fluid from a cyst or an extract of the membrane forming the wall of a cyst. It appears that the fluids obtained from different specimens of cyst differ considerably in antigenic properties. Vas<sup>5</sup> considers that fluid from cysts found in cattle is satisfactory, but Dobrotin<sup>6</sup> and Silber and Chmelnitzky<sup>7</sup> hold that human cyst-fluid must be used. Hahn<sup>8</sup> states

<sup>1</sup> See Nice, MacLester, and Torrance, *Journ. Amer. Med. Assoc.*, March 25, 1911; Cole and Winthrop, *ibid.*, Jan. 17, 1911.

<sup>2</sup> *Compt. Rend. Soc. Biol.*, 1907, lxii, 1198.

<sup>3</sup> *Lancet*, 1908, i, 1338.

<sup>4</sup> *Gaz. degli Ospedali.*, 1906, p. 1907.

<sup>5</sup> *Wien. med. Woch.*, 1911, p. 251.

<sup>6</sup> *Berl. klin. Woch.*, 1910, No. 28.

<sup>7</sup> Abstr. in *Zeitschr. f. Immunitätsforsch.*, 1913, p. 800.

<sup>8</sup> *Münch. med. Woch.*, 1912, p. 1483.

that the fluid is of no value as antigen, and that a watery extract of the cyst-wall itself must be employed. Israel<sup>1</sup> finds that the fluid, and watery or alcoholic extracts of the cyst, are equally efficient. Weinberg<sup>2</sup> recommends that the test should be performed first according to the method of Stern,<sup>3</sup> with fresh human serum containing complement, and then by his own method, with addition of guineapig's serum. Zappelloni and Ricciuti<sup>4</sup> found positive reactions in all of 33 cases, and other writers record useful results. The reaction may remain positive for a considerable length of time after the cyst has been removed by surgical interference, so that the test is of no use as a means of recognizing relapses. A positive reaction may occur in patients infected with *Tenia saginata* (Hahn). Meyer<sup>5</sup> denies that the test has any appreciable value, finding positive reactions in healthy subjects and negative in the infected.

<sup>1</sup> *Zeitschr. f. Hyg.*, 1910, lxvi., Heft 3.

<sup>2</sup> *Compt. Rend. Soc. Biol.*, 1909, lxvi. 816.

<sup>3</sup> *Zeitschr. f. Immunitätsforsch.*, 1909, p. 422.

<sup>4</sup> *Abst. ibid.*, 1910, p. 678.

<sup>5</sup> *Berl. klin. Woch.*, 1910, No. 28.



# INDEX

## A

- Abrin, 5, 437
- Achorion quinekeanum, 347
- Acue, 382
  - indurata, 383
- Addiment, 10
- Agglutination, 13, 76
  - diagnostic use of, 76
  - group-, 81
  - nature of, 15
  - of *B. diphtheriæ*, 136
  - of *B. dysenterici*, 258
  - of *B. mallei*, 204
  - of *B. pestis*, 206
  - of *B. tuberculosis*, 289
  - of *B. typhosus*, 230
  - of blood-corpuscles, 13
  - of meningococcus, 385
  - of *Micrococcus melitensis*, 81-396
    - of pneumobacillus, 373
    - of pneumococcus, 373
    - of Shiga's bacilli, 81
    - of *Spirillum obermeieri*, 420
    - of staphylococci, 382
    - of streptothrix, 347
    - of *Vibrio cholerae*, 221
    - persistence of, 82, 234
    - test, 76
    - — macroscopic, 79
- Agglutinins, 14, 15
- Aggressins, 20
- Albuminuria and tuberculin, 314
  - due to serum, 130
- Alexines, 2, 10 (*see* Complement)
- Allergy (*see* Anaphylaxis)
- Amboceptor, 10
- Anæmia after vaccination, 191
  - pernicious, 358, 419
- Anaphylaxis, 20
- Angina redux, 128
- Anthrax, 201
- Antiabrin serum, 437
- Autianaphylaxis, 21
- Autibacterial serum, 6, 43
- Antibodies, 10, 11
  - excess of, 37
  - source of, 33, 35
- Antibody, use of word 12
- Autigen, 12
- Antimeristem, 429
- Antimony, preparations of, 413
- Antiopsonins, 20
- Antiparatyphoid inoculation, 255
- Antiphthisin, 284
- Antirabic serum, 179
  - vaccination, 171
    - effects of, 174
    - results of, 175
- Antiscarlatinal serum, 382
- Antistreptococcic serum, 342, 351
  - ill effects of, 359
  - in cellulitis, 356
  - in enteric fever, 254
  - in erysipelas, 356
  - in gangrenous stomatitis, 358
  - in local collections of pus, 359
  - in pernicious anæmia, 358
    - in puerperal fever, 354
    - in purpura, 359
    - in scarlatina, 370
    - in septicæmia, 356
    - in small-pox, 199
    - in tuberculosis of the lungs, 342
    - in ulcerative endocarditis, 356
    - Marmorek's, 352
    - value of, 360
- Antitoxin, diphtherial, 98
  - administration of, 116
  - ill effects of, 126
  - refined, 108
  - standard of, 100

Antitoxin, diphtherial, statistics of, 110  
 — strength of, 107  
 — unit of, 100  
 — use of fresh, 48  
 — in prophylaxis, 118  
 — value of, 108  
 — in paralysis, 124  
 — hay-fever, 399  
 — morphine, 436  
 — tetanus, 142  
 Antitoxins, action of, 25, 26  
 — chemical nature of, 5  
 — discovery of, 4  
 — formation of, 25  
 Antitryptic value of serum, 423  
 Antityphoid extract (Jez's), 244  
 — inoculation, 246  
 — serum, 235, 242  
 Antivenene, 163  
 — dose of, 166  
 — in leprosy, 345  
 — standardization of, 166  
 Appendicitis, 363  
 Aronson's serum, 352  
 Arseno-benzol, 414  
 Arthigon, 393  
 Atoxyl, 412  
 Attenuation of bacteria, 59, 329  
 Autolysins, 11  
 Autumn catarrh, 401

## B

Bacillosine, 279  
 Bacillus anthracis, 201  
 — coli communis, 262  
 — diphtheriæ, 94  
 — dysentericæ, 257  
 — emulsion, 273, 277  
 — enteritidis, 254  
 — lepræ, 345  
 — mallei, 203  
 — of whooping-cough, 266  
 — paratyphosus, 254  
 — pestis, 206  
 — pyocyaneus, 264  
 — tetani, 138  
 — tuberculosis, 270  
 — typhosus, 229  
 Bactericidal serum, 6  
 Bacteriolysis, 7  
 — modification of, in tissues, 31  
 Benzidine colours, 413

Beraneck's tuberculin, 286  
 Blood, human, test for, 90  
 Boils, 383  
 Botulism, 268  
 Broncho-pneumonia, 379, 380  
 Bruschetтини's sero-vaccine, 280

## C

Calf-lymph, 184  
 Calmette's antivenene, 163  
 — ophthalmic reaction, 309  
 — tuberculin, 287  
 Cancer (*see* Tumours)  
 Cancroidiu, 429  
 Cancroiu, 430  
 Cancrum oris, 132  
 Carbuncle, 382  
 Carriers, diphtheria, 135  
 — typhoid, 254  
 Catarrh, nasal, 403  
 Catarrhal affections, 398  
 Cellulitis, 356  
 Cerebral emulsion in tetanus, 155  
 Chantemesse's serum, 235  
 Cheloid after vaccination, 192  
 Chemotherapy, 412  
 — of pneumouia, 380  
 — of relapsing fever, 421  
 — of syphilis, 412  
 Chloroform-lymph, 185  
 Cholera, 220  
 — diagnosis of, 221  
 — passive immunity to, 228  
 — vaccination against, 222  
 Chorea, 368  
 Cobra-hæmolysin, 160  
 Cobra-lecithide, 161  
 Cocci, affections due to, 373  
 Coley's fluid, 424, 433, 441  
 Colitis, 262  
 Complement, 8, 10  
 — chemical nature of, 34  
 — deficiency of, 38  
 — deviation of, 38  
 — distribution of, 34  
 — fixation of, 38  
 — plurality of, 35  
 — source of, 34  
 Conductor, 106  
 Conjunctiva, diphtheria of, 131  
 Contratoxin, 342  
 Convalescents, serum of, in meningitis, 387



Convalescents, serum of, in pneumonia, 376  
 ——— in scarlatina, 371  
 Copula, 8, 10  
 ——— excess of, 38  
 ——— source of, 35  
 Corneal ulcer, 376  
 Coryza, 403  
 Cow-pox, identity of, with small-pox, 183  
 Croton, 5  
 Cutaneous eruptions due to serum, 128  
 ——— reaction, 308, 355  
 ——— in gonorrhœa, 391  
 ——— in puerperal fever, 355  
 ——— in syphilis, 410  
 ——— in tuberculosis, 308  
 ——— in tumours, 423  
 Cyclaster scarlatinalis, 369  
 Cytase, 10  
 Cytolysins, 12  
 Cytolysis, 11  
 Cytolytic serum for tumours, 429  
 Cytoryctes variolæ, 182

D

Death from serum injections, 57, 127  
 Denys' tuberculin, 285  
 Desmon, 10  
 Diagnosis by complement-fixation, 89, 295, 423  
 ——— by opsonins, 83  
 ——— by physico-chemical methods, 91  
 ——— by serums, 289  
 ——— by toxins, 91  
 ——— by tuberculin, 299  
 ——— by vaccines, 391  
 ——— of tumours, serological, 423  
 Diarrhœa, summer, 261  
 Diphtheria, 94  
 ——— active immunization to, 121  
 ——— agglutination in, 136  
 ——— antibacterial serum for, 134  
 ——— as complication, 132  
 ——— bacillus of, 94  
 ——— conjunctival, 131, 135  
 ——— diagnosis of, 136  
 ——— nasal, 133  
 ——— persistent faucial, 75  
 ——— toxins of, 96

2 D

Diphtheria, vaccine treatment of, 135  
 Diphtherial antitoxin, 98  
 ——— administration of, 116  
 ——— conclusions as to, 136  
 ——— discovery of, 4  
 ——— dose of, 121  
 ——— ill effects of, 126  
 ——— in cancrum oris, 132  
 ——— in conjunctivitis, 131  
 ——— in ear-disease, 132  
 ——— in Graves's disease, 441  
 ——— in meningitis, 387  
 ——— in ozæna, 133  
 ——— in paralysis, 124  
 ——— in pneumonia, 379  
 ——— in septic conditions, 359  
 ——— in whooping-cough, 268  
 ——— local use of, 135  
 ——— manufacture of, 98  
 ——— post-diphtheritic paralysis and, 124  
 ——— prophylactic use of, 118  
 ——— standardization of, 100  
 ——— unit of, 100  
 Diplococcus gonorrhœæ, 389  
 ——— hemipholus, 128  
 ——— intracellularis, 385  
 ——— pneumoniæ, 373  
 Dixon's tuberculin, 281  
 Dmego, 393  
 Dunbar's serum, 401  
 Dysentery, 257  
 ——— agglutination test in, 81, 258  
 ——— serum treatment of, 259  
 ——— vaccine prophylaxis of, 260  
 ——— treatment of, 261

E

Ear-disease, post-scarlatinal, 132  
 Eel, serum of, 11  
 Ehrlich's theory of immunity, 22  
 Emmerich and Scholl's serum, 427  
 Empyema, 378  
 Emulsion of tubercle bacilli, 273, 276  
 Endocarditis, staphylococcal, 382  
 ——— ulcerative, 356, 363  
 Endocomplement, 161  
 Entamœba dysentericæ, 257  
 Enteric fever, 229  
 ——— agglutination-test for, 76

- Enteric fever, antibacterial serum  
 in, 241, 242  
 ——— antistreptococcic serum  
 in, 254  
 ——— antitoxic serum in, 235  
 ——— complications of, 229  
 ——— diagnosis of, 230  
 ——— Jez's extract in, 244  
 ——— ophthalmic reaction, 235  
 ——— vaccination against, 246  
 ——— vaccine treatment of,

252

- Euteritis, infantile, 261  
 Epilepsy, 160  
 Epiphanin reaction, 92  
 Eruptions, cutaneous, 128  
 Erysipelas after vaccination, 190  
 ——— in tumours, 424  
 ——— serum treatment of, 356  
 ——— vaccine treatment of, 363  
 Erythema, bullous, 384  
 ——— from serum-injections, 128  
 Exophthalmic goitre, 437

## F

- Ferments, protective, 21  
 Ficker's diagnostic, 80  
 Fixative, 10  
 Frambæsia, 409, 418  
 Friedmann's tuberculin, 281  
 Furunculosis, 383

## G

- Gabritschewsky's vaccine, 372  
 Galy, 418  
 Gastric ulceration, 435  
 General paralysis, 408, 419  
 Glanders, 203  
 Glands, swollen, due to serum,  
 128  
 Gleet, chronic, 394  
 Globulins, relation of, to agglu-  
 tinins, 17  
 ——— to antitoxins, 5  
 Gonococcus, 389  
 Gonorrhœa, 75  
 ——— complement-fixation in, 390  
 ——— cutaneous reaction in, 391  
 ——— serum treatment of, 391  
 ——— vaccine diagnosis of, 391  
 ——— treatment of, 392  
 Gonotoxin, 389  
 Graves's disease, 437  
 Group-reactions, 17

## H

- Hæmatoëthyroidine, 440  
 Hæmoglobinuria, 442  
 Hæmolysis, 7  
 ——— due to snake-venom, 160  
 Hæmolytic skin-reaction, 423  
 Hæmophilia, 435  
 Hæmoptysis, 435  
 Haffkine's cholera-vaccine, 222  
 ——— plague-prophylactic, 207  
 Haptophore, 25  
 Hay-fever, 398  
 Hermann-Perutz reaction, 93  
 Herzheimer reaction, 415  
 Hodgkin's disease, 442  
 Hogen's antirabic vaccination, 174  
 Horse serum, normal, 434  
 Humoral theory, 3  
 Hydatid disease, 444  
 Hydrophobia, 168  
 ——— incubation of, 170  
 ——— Pasteur treatment of, re-  
 sults of, 175  
 ——— serum treatment of, 179  
 ——— vaccination against, 171  
 ——— virus of, 169  
 Hypersensibility, 20

## I

- Idiosyncrasy to serums, 130  
 Immune body, 10  
 Immunity, acquired, 30  
 ——— active, 30  
 ——— forms of, 29  
 ——— maternal transmission of, 32  
 ——— natural, 29  
 ——— passive, 30  
 ——— theory of, 22  
 Immun Körper, 341  
 Incubation period of toxins, 28  
 Infantile enteritis, 261  
 Infants, administration of serum  
 to, 49  
 Inheritance of immunity, 32  
 Inoculation, autityphoid, 246  
 ——— site of, 74  
 ——— small-pox, 1  
 Intermediary body, 10  
 Intracerebral injection of serum,  
 52, 149  
 Intramine, 418  
 Intramuscular injection of se-  
 rum, 55

Intraneural injection of serum,  
150  
Intrathecal injection of serum,  
54, 148, 387, 388  
Intravenous injection of serum,  
51, 116, 148  
Iron-tuberculin, 288  
Isolysins, 11  
Italian method of antirabic in-  
oculation, 171, 180

## J

Jennerization, 59  
Jez's antityphoid extract, 244  
Joints, pains in, 130

## K

Kala-azar, 443  
Keratitis, ulcerative, 376  
Kharsivan, 418  
Klausner's reaction, 93  
Koch's emulsion of bacilli, 273,  
276  
— tuberculin, 271  
Kolle's cholera vaccine, 223

## L

Lactic-acid bacilli, use of, 74  
Lanz's milk treatment, 438  
Laryngitis, tubercular, 326  
Lecithide, cobra-, 161  
Leprosy, 345, 409  
Leuchæmia, 441  
Leucocidin, 381  
Leucocytes as source of comple-  
ment, 3, 33  
— — of copula, 35  
— in agglutination, 17  
— protective action of, 2  
Leucolysis, 161  
Leucolytic serum, 441  
Living tissues, influence of, on  
bacteriolysis, 31  
Local use of serum, 50  
Lozenges, serum in, 135  
Luetin reaction, 410  
Lumbar puncture, 54  
Lupus vulgaris, 322  
Lustig and Galeotti's vaccine, 211  
Lustig's serum, 216  
Lymph, vaccine, supply of, 193  
— — preparation of, 184

## M

Malaria, 409, 419  
Malignant disease (*see* Tumours)  
Mallein, 204  
Malta fever, 81, 395  
Mandelbaum's reaction, 235  
Maragliano's serum, 337  
Marmorek's serum, antistrepto-  
coccic, 352  
— — antitubercular, 339  
Maternal transmission of im-  
munity, 32  
Mediator, 10  
Mediterranean fever, 81, 395  
Meiostagmin reaction, 92  
Meningitis, cerebro-spinal, 385  
— — serum treatment of, 386  
— — vaccine treatment of,  
388  
Meningococcus, 385  
Menzer's serum, 366  
Micrococcus catarrhalis, 403  
— melitensis, 395  
— neoformans, 428, 432  
— rheumaticus, 365  
Milk of thyroidectomized ani-  
mals, 438  
— transference of immunity  
by, 32  
Morbilliform rash, 129  
Morphine antitoxin, 436  
Mouth, absorption of antitoxin  
by, 32  
— administration of serum by,  
117, 360, 439  
— — of tuberculin by, 336

## N

Nasal diphtheria, 133  
Nastin, 346  
Negative phase, 69  
Neosalvarsan, 417  
Nephritis due to serum, 130  
Neurotoxin, 162  
Novo-arseno-benzol, 417

## O

Operations, normal serum in, 434  
Ophthalmic reaction, 235, 309, 402  
— — enteric, 235  
— — tubercular, 309  
— use of antiabrin, 437  
Opsonin, 3, 18

Opsonin, diagnosis by, 83  
 — estimation of, 84  
 Opsonin-index after vaccination,  
 69  
 — meningococcic, 389  
 — tubercular, 83  
 — value of estimation of, 72,  
 86  
 Optochin, 380  
 Oral administration of serum, 117  
 — — of tuberculin, 336  
 Oriental sore, 443  
 Otitis media, pneumococcic, 378  
 Overproduction theory, 25  
 Oxytuberculin, 287  
 Ozæna, 133

## P

Paralysis, diphtheritic, 124  
 — general, 408, 419  
 Paramelitensis fever, 395  
 Paramœcium caudatum, 182  
 Paratyphoid infections, 254  
 Pasteurization, 59  
 Pasteur's vaccine for hydro-  
 phobia, 171  
 Pellagra, 443  
 Percutaneous reaction, 308  
 Periorbitis, 383  
 Peritonitis, acute diffuse, 75  
 — pneumococcic, 378  
 — tubercular, 329  
 Pernicious anæmia, 358  
 Pfaundler's reaction, 79  
 Pfeiffer's experiment, 9, 222  
 Phagocytosis, 2  
 Phase, positive and negative, 69  
 Phlogosin, 382  
 Phylacogen, 69  
 Piorkowsky's tuberculin, 282  
 Plague, 206  
 — agglutination in, 206  
 — Haffkine's prophylactic, 207  
 — Lustig-Galcotti vaccine, 211  
 — Lustig's serum, 216  
 — Terni-Bandi vaccine, 211  
 — Yersin's serum, 213  
 Pneumobacillus, 373  
 Pneumococcic affections, 373  
 — empyema, 378  
 — endocarditis, 375  
 — otitis, 378  
 — peritonitis, 378

Pneumonia, chemotherapy of, 380  
 — serum treatment of, 374  
 — vaccine treatment of, 377  
 Pollantin, 402  
 Porges' reaction, 92  
 Positive phase, 69  
 Postdiphtheritic paralysis, 124  
 Precipitation, 12  
 — tests, 90, 298, 444  
 Precipitins, 12  
 Préparateur, 10  
 Preparation of serums, 41, 43  
 Prophylactic, Haffkine's plague,  
 207  
 — immunization in diphtheria,  
 121  
 — use of antitoxin, 118, 121, 151  
 — — of serum, 216  
 — — of vaccines, 59, 203, 260,  
 378, 389, 397, 403, 404  
 — von Ruck's, 278  
 Prototoxoid, 103  
 Protozoa, diseases due to, 405  
 Puerperal fever, 354  
 Puncture, lumbar, 54  
 Purpura hæmorrhagica, 359, 435  
 Pus, collections of, 359  
 Pyæmia, pneumococcic, 378  
 — staphylococcic, 383  
 Pyorrhœa alveolaris, 384

## R

Rabies (*see* Hydrophobia)  
 Rashes, antitoxin, 128  
 Reaction, epiphanin, 92  
 — hæmolytic skin-, 423  
 — Hermann-Perutz, 93  
 — Herxheimer's, 415  
 — Klausner's, 93  
 — luetin, 410  
 — Mandelbaum's, 235  
 — meiotagmin, 92  
 — ophthalmic, 235, 309, 402  
 — percutaneous, 308  
 — Pfaundler's, 79  
 — Porges', 92  
 — Rivalta's, 35  
 — Wassermann's, 405, 407  
 Receptors, 24  
 — cholera, 227  
 Rectum, administration of serum  
 by, 117, 358  
 Relapsing fever, 409, 420

Resistance, acquired, 1  
 — of tissue-cells, 3  
 Revaccination, 187  
 Rheumatism, 365  
 Rhinitis, 75, 133  
 Ricin, 5  
 Riggs's disease, 384  
 Ringworm, 347  
 Rivalta's reaction, 35  
 Rodagen, 438  
 Rosenbach's tuberculin, 282  
 Roux's point, 53

## S

Salvarsan, 414  
 — administration of, 414  
 — excretion of, 415  
 — ill effects of, 415  
 — in anthrax, 203  
 — in dysentery, 257  
 — in hydrophobia, 181  
 — in kala-azar, 443  
 — in pellagra, 443  
 — in small-pox, 200  
 — in syphilis, 412, 414  
 — in yaws, 418  
 — intrathecal injection of, 419  
 — substitutes for, 417  
 Sarcoma, Coley's fluid in, 426  
 — Wassermann reaction in, 409  
 Scarlet in, 370  
 Scarlatina, 369, 409  
 — serum treatment of, 370  
 — vaccine treatment of, 372  
 Scarlatinal ear-disease, 132, 371  
 Scarlatiniform rash, 129  
 Sclavo's serum, 201  
 Sedimentation test, 79  
 Sensitized corpuscles, 11  
 — vaccines, 67  
 Sensitizing substance, 10, 11  
 Septic conditions, diphtherial  
 antitoxin in, 359  
 Septicæmia, 356  
 — gonorrhœal, 392  
 Sero-vaccine, Bruschetti's, 280  
 Serum, administration of, 41, 46,  
 50  
 — antiabrin, 437  
 — antiamarillic, 266  
 — antianthrax, 201  
 — antibacterial, 6, 43  
 — anticancerous, 427  
 Serum, anticrotalis, 165  
 — antidysenteric, 259  
 — antileptous, 345  
 — antimeningococcic, 386  
 — antiplague, 213, 216  
 — antipneumonic, 374, 388  
 — antirabic, 179  
 — antirheumatic, 366  
 — antiscarlatinal, 370  
 — antistaphylococcic, 382  
 — antistreptococcic, 342, 351  
 — antitoxic, preparation of, 6,  
 41  
 — antitubercular, 337  
 — antityphoid, 235  
 — bactericidal, 6  
 — Carrasquilla's, 345  
 — Chantemesse's, 235  
 — cytolytic, 429  
 — Deutsch's, 203  
 — dose of, 47  
 — Doyen's, 428  
 — Dunbar's, 401  
 — Emmerich and Scholl's, 427  
 — for *B. coli*, 263  
 — for *B. pyocyaneus*, 264  
 — for Graves's disease, 440  
 — for Mediterranean fever, 396  
 — for relapsing fever, 421  
 — for syphilis, 411  
 — for whooping-cough, 267  
 — hæmolytic action of foreign,  
 11  
 — homologous, 44  
 — horse-, normal, 434  
 — idiosyncrasy to, 130  
 — ill effects of, 55, 126, 359  
 — immune, 12, 19  
 — leucocytic, 441  
 — Leuriaux's, 267  
 — local use of, 50  
 — Lustig's, 216  
 — MacConkey's, 218  
 — Maragliano's, 337  
 — Marmorek's, 339, 352  
 — Mendez's, 203  
 — Menzer's, 366  
 — Moser's, 370  
 — of convalescents, 371, 376,  
 387  
 — of immune cattle in small-  
 pox, 199  
 — of thyroidectomized ani-  
 mals, 439

- Serum, oral administration of, 50  
 — polyvalent, 44  
 — preparation of, 41  
 — protective power of, 2  
 — rashes, 57  
 — rectal administration of, 117, 340, 358  
 — Römer's, 375  
 — Schmidt's, 429  
 — Sclavo's, 201  
 — sickness, 57  
 — standardization of, 45  
 — testing of, 44  
 — thyrotoxic, 440  
 — treatment, theory of, 41  
 — use of fresh, 48  
 — — of warm, 131  
 — Wlaeff's, 427  
 — Yersin's, 213  
 Shock, anaphylactic, 20  
 Side-chain hypothesis, 23  
 Site of inoculation, 74  
 Small-pox, 182  
 — complications of, 183  
 — identity of, with cow-pox, 183  
 — inoculation against, 1  
 — modified, 198  
 — serum treatment of, 199  
 — vaccination against, 183  
 Snake-bite, 157  
 Snakes, varieties of poisonous, 157  
 Snake-venom, 158  
 Specific nature of antitoxins, 5  
 Spirillum cholerae, 220  
 — obermeieri, 420  
 Spirochæta obermeieri, 420  
 — pallida, 405  
 Sporotrichum beurmanni, 347  
 Stages of phthisis, 318  
 Staphylococci, affections due to, 381  
 — toxins of, 381  
 Stimulins, 18  
 Stomatitis, gangrenous, 358  
 Streptococci, affections due to, 349  
 Streptococcic vaccine, 362  
 Streptococcus faecalis, 364  
 — pyogenes, 351  
 — rheumaticus, 365, 366  
 Streptotrichosis, 347  
 Subcutaneous injection of serum, 50  
 Subdural injection, 148  
 Substance sensibilisatrice, 10, 11  
 Supersensibility, 20  
 Sycosis, 383  
 Syncoccus, 393  
 Syphilis, 405  
 — chemotherapy of, 412  
 — from vaccination, 190  
 — serum reaction in, 405  
 — — treatment of, 411  
 — Wassermann reaction in, 405, 408  
 Syringe for serum, 48  
 — for vaccines, 73  
 — sterilization of, 49
- T
- Tabes dorsalis, 408  
 Tebean, 289  
 Terni and Bandi's vaccine, 211  
 Test, agglutination, 14, 76, 79  
 — Calmette's, 309  
 — cutaneous, 308, 355  
 — precipitation, 90  
 — sedimentation, 79  
 — von Pirquet's, 307  
 — Wassermann's, 408  
 — Wassermann-Uhlenhuth, 90  
 — Widal's, 76, 231  
 — — sources of error in, 231  
 — (see also Reaction)  
 Tetanine, 141  
 Tetanolysin, 141  
 Tetanus, 138  
 — antitoxin, 142  
 — — administration of, 147, 156  
 — — dose of, 151  
 — — ill effects of, 154  
 — — preparation of, 142  
 — — prophylactic use of, 151, 156  
 — — standardization of, 142  
 — — statistics of, 146, 152  
 — — unit of, 143  
 — — use of, 145  
 — — value of, experimental, 144  
 — cerebral emulsion in, 155  
 — from vaccination, 190  
 — toxins of, 29, 140  
 Thyroidectin, 440  
 Tissues, fixation of toxins hy, 35

- Tissues, influence of, on bacteriolysis, 34  
 — resistance of, 3  
 Tonsillitis due to serum, 128  
 Toxins, absorption of, by mouth, 33  
 — action of, 27, 28  
 — as curative agents, 68, 426  
 — as diagnostic agents, 91  
 — incubation period of, 28  
 — interaction with antitoxins, 101  
 — of *B. coli*, 263  
 — of *B. diphtheriæ*, 96  
 — of *B. dysenteriæ*, 257  
 — of *B. icteroides*, 265  
 — of *B. mallei*, 204  
 — of *B. pyocyaneus*, 264  
 — of *B. tetani*, 140  
 — of *B. tuberculosis*, 271  
 — of *B. typhosus*, 230  
 — of gonococcus, 389  
 — of hay-fever, 399  
 — of staphylococci, 381  
 — of streptococci, 351  
 — of *V. cholerae*, 220  
 Toxines and toxoids, 103  
 Toxophore, 25  
 Treponema pallidum, 405  
 Tricophyton holosericum, 282  
 Trixidine, 413  
 Trypan-red and -blue, 413  
 Tuberal, 279  
 Tuberculin, 68, 271  
 — action of, 273  
 — administration of, 332  
 — alkalium, 273  
 — alt, 271  
 — Beraneck's, 286  
 — bovine, 278  
 — by-effects of, 314  
 — Calmette's, 287  
 — choice of, 333  
 — composition of, 272  
 — contraindications, 304  
 — Denys', 285  
 — diagnostic use of, 83, 299  
 — Dixon's, 281  
 — dosage of, 334  
 — Friedmann's, 281  
 — in disease of bones and joints, 327  
 — in genito-urinary disease, 329  
 Tuberculin in leprosy, 346  
 — in leucæmia, 441  
 — in lupus, 322  
 — in ophthalmic disease, 328  
 — in pulmonary tuberculosis, 314  
 — in tubercular laryngitis, 326  
 — iodized, 288  
 — iron, 288  
 — modifications of, 273, 278  
 — new, 273  
 — Oberer 273  
 — old, or original, 271  
 — oxy-, 287  
 — Piorkowsky's, 282  
 — Rosenbach's, 282  
 — rückstand (T.R.), 273  
 — sanocalcin, 288  
 — therapeutic use of, 312  
 — varieties of, 273  
 — v. Ruck's, 278  
 Tuberculocidin, 284  
 Tuberculol, 285  
 Tuberculomuciu, 283  
 Tuberculoplasmin, 279  
 Tuberculosis, 270  
 — agglutination test in, 289  
 — complement-fixation in, 295  
 — nature of immunity to, 311  
 — pulmonary, 160, 314, 435  
 — serum diagnosis of, 289  
 — — treatment of, 337  
 — surgical, 321  
 — vaccination against, 329  
 — vaccine treatment of, 332  
 Tuberculo-toxoidin, 287  
 Tulase, 280  
 Tumours, malignant, 422  
 — — Coley's fluid in, 424  
 — — diagnosis of, 423  
 — — serum treatment of, 427  
 — — vaccine treatment of, 429, 432  
 Typhoid fever (*see* Enteric fever)
- U
- Ulceration, gastric, 435  
 Ulcerative endocarditis, 356, 363  
 Ulcus serpens corneæ, 376  
 — tropicum, 409, 419  
 Undulant fever (*see* Mediterranean fever)  
 Uuit, antitoxic, 100



Urethritis, gonococcal, 389  
 Urine, suppression of, 130  
 Urticarial rash, 130

## V

Vaccination, bacterial, 59  
 ——— anticholera, 222  
 ——— antiplague, 207  
 ——— antirahic, 171  
 ——— antitubercular, 329  
 ——— antityphoid, 246  
 ——— phenomena of, 69  
 ——— small-pox, 183  
 ——— insusceptibility to, 192  
 ——— phenomena of, 187  
 ——— protection by, 193, 197  
 ——— risks of, 190  
 ——— statistics of, 194  
 ——— subcutaneous, 186  
 ——— technique of, 185, 187  
 ——— theory of, 183  
 Vaccine, anthrax, 203  
 ——— cholera, 222, 223  
 ——— lymph (*see* Lymph)  
 ——— origin of term, 59  
 ——— treatment of coli-infections,  
     263  
 ——— of diphtheria, 135  
 ——— of dysentery, 261  
 ——— of enteric fever, 252  
 ——— of glanders, 204  
 ——— of gonococcal infections,  
     392  
 ——— of hay-fever, 402  
 ——— of Hodgkin's disease, 442  
 ——— of leprosy, 346  
 ——— of Mediterranean fever,  
     397  
 ——— of meningitis, 388  
 ——— of nasal catarrh, 403  
 ——— of new growths, 432  
 ——— of pneumococcal infec-  
     tions, 377  
 ——— of scarlatina, 372  
 ——— of staphylococcal infec-  
     tions, 382  
 ——— of streptococcal infec-  
     tions, 362  
 ——— of tuberculosis, 332  
 ——— of tumours, 432  
 ——— theory of, 59

Vaccines, 59  
 ——— administration of, 72  
 ——— as treatment, 62  
 ——— autogenous, 64  
 ——— autolysed, 394  
 ——— Haffkine's plague, 207  
 ——— Lustig and Galeotti's, 211  
 ——— preparation of, 63  
 ——— prophylactic, use of, 59  
 ——— sensitized, 67, 377, 384, 394  
 ——— standardization of, 66  
 ——— stock, 64, 383, 393  
 ——— Terni and Bandi's, 211  
 ——— vaccinal, 184  
 ——— Wright's antityphoid, 246  
 Vaccinia, 182  
 ——— gangrenosa and hæmorrhagica, 191  
 ——— generalized, 191  
 Variola (*see* Small-pox)  
 Venom, snake, action of, 159  
 ——— nature of, 158  
 Vibrio cholerae, 220  
 Vulvo-vaginitis, 379

## W

Wassermann reaction, 405  
 ——— in syphilis, 405, 408  
 ——— quantitative, 407  
 ——— in various conditions,  
     409  
 ——— modifications of, 408  
 ——— significance and varia-  
     tions of, 409  
 Wassermann-Uhlenhuth test, 90  
 Whooping-cough, 266  
 Widal's test, 76, 231  
 ——— sources of error in, 231  
 ——— value of, 80, 234  
 Wlaeff's serum, 427  
 Wright's antityphoid vaccine, 246

## Y

Yaws, 409, 418  
 Yellow fever, 265  
 Yersin's serum, 213

## Z

Zone of inhibition, 17



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